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Reports on a symposium held at Ribeirao Preto, Brazil, in November, 1973, on improving the production and nutritional quality of beans and other legumes, which are widely used as staple foods. The 22 participants were nutritional and food science specialists from Latin America, the U.S., Asia, and Africa. These proceedings of the symposium present the 25 papers delivered on the subjects of (1) legumes as animal feed; (2) storage, processing, and nutritive value of legumes; and (3) utilization of common beans and other legumes as human food. Also includes summaries of workshop sessions on these topics. Three of the major recommendations are that the Green Revolution in cereal crops be extended to legumes, that storage facilities for large and small producers be greatly increased, and that research on nutritional qualities of legumes be greatly expanded.

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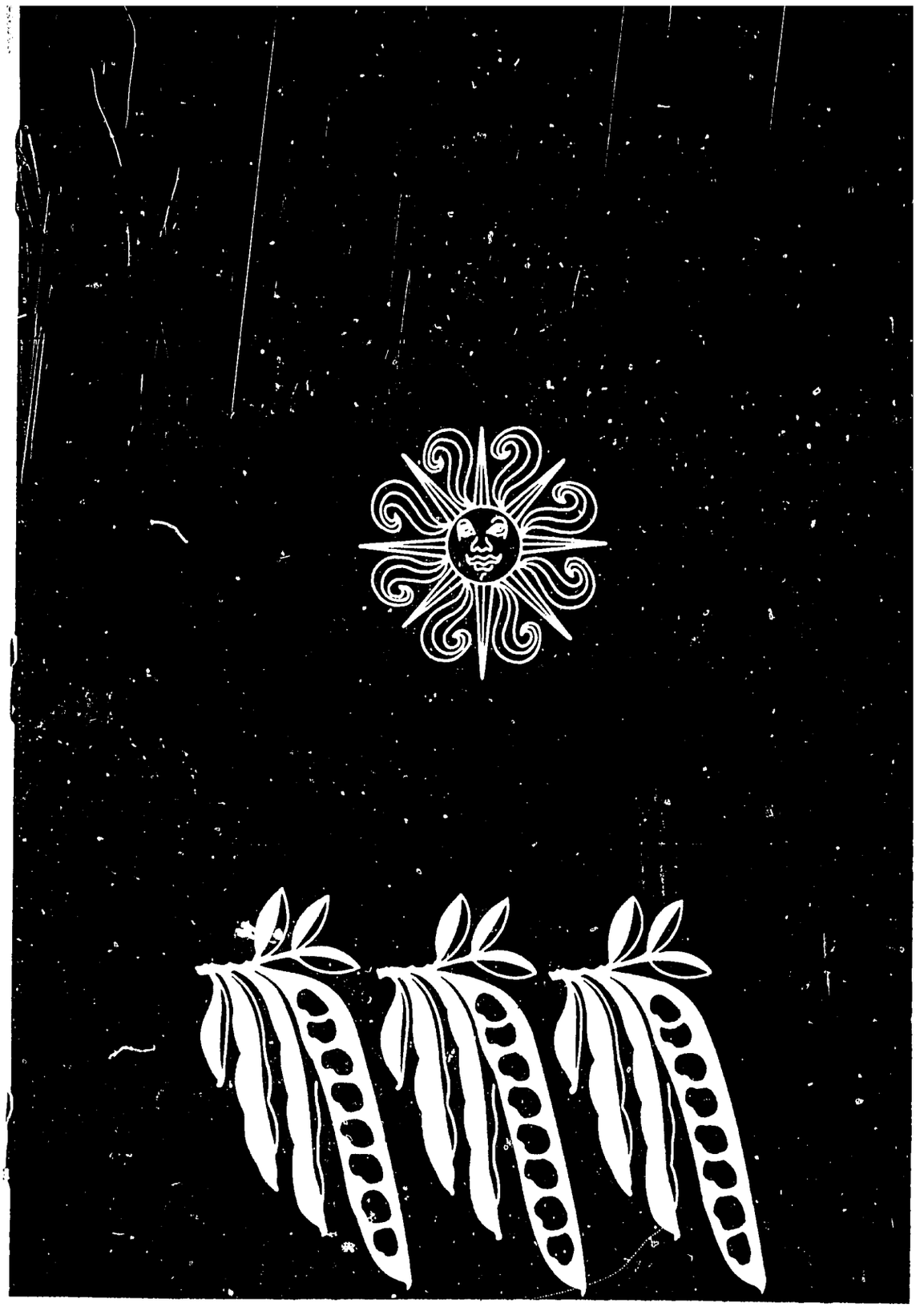

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Nutritional aspects of common beans and other legume seeds as animal and human foods

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Preface

The common beans are a gift of Latin America to the world. The wild ancestral bean species has been identified at various locations ranging from Argentina to México. Black bean seeds, dating back to over 3.000 years, but similar to varieties still very much in use in some parts of northern Latin America, have been recovered from archeological deposits in México and Perú. Beans are consumed for breakfast, lunch and dinner together with corn or rice, and bean broth is used as weaning food by large populations of this region. In many of the less developed countries beans and others legumes together with cereals constitute the staple diet for the poorest classes, and contribute very significantly to their protein intake. In contrast, in the technically more advanced nations legume consumption is of little importance and until recently there has been scant interest in these countries in problems such as the improvement of production and nutritional quality, the handling and technology of legumes.

A demostration of the growing concern in this subject is the symposium on beans and other legumes held November 6-9, 1973 at Ribeirao Preto.

During the meeting, morning sessions were dedicated to the presentation and discussion of invited papers. In the afternoon, round-table seminars were organized during which three subjects were freely descussed in groups of about 12-15 participants. Summaries of the results of these workshops as well as the papers presented and of an extra session on nutritional qualities of beans are included in the present volume, which by no means provides an exhaustive or even balanced treatment of the subject. It may serve, nevertheless to the purpose of promulgating some new results and ideas and hopefully will stimulate further research in this field.

Werner G. Jaffé, Ph. D.

Opening remarks

It is a pleasure and an honor for our country and for Ribeirao Preto to be the host of an international meeting on legume seeds.

Brazil, the largest producer of common beans in the world has experienced tremendous growth in the production of soybeans.

We certainly have to be aware of the importance of legume seeds as food for both humans and for animals. It is our hope that some of the attention given to the cereals in the last several years could also be focused on the legume seeds. Legumes are without doubt a good source of protein and we have to work to improve their quality and to increase the supply.

I am sure we will be hearing about developments in this area, from what is going on in this subject from several parts of the world.

Three main topics will be covered in this meeting:

a) Common beans and other legumes as animal feed;
b) Processing, storage and nutritive value of common beans and other legumes; and
c) Utilization of common beans and other legumes as human food.

It is expected that the reports presented in this meeting will help to fill a gap. The round table discussions will give the opportunity for a more informal and in-depth exchange of ideas, suggestions for future work in different areas. An extra session has been included in the program on the way how collaborative research and applied projects on legumes could be planned and carried out in the future. We are very appreciative and optimistic about this possibility.

May I now point out two aspects I consider of great importance in this meeting:

First: The intersectorial and multidisciplinary trend. People working in different fields, who are dealing with a common problem, food, should work together more often.

Second: A general problem, I've noticed at several international meetings. There is never sufficient time for a discussion of the broader view of the subjects under study and more contact among the participants. We hope to do this here. In the morning we will hear papers and in the afternoon we will get together in round table discussions. These have been planned in order to give a greater opportunity for participation of everyone present.

Finally, it must also be said that this meeting would not have been possible without the efforts of the local teams and the support of the Agency for International Development (USAID).

The possibility of different activities during my year as Visiting Professor at Cornell University was an important factor in the organization of this conference.

The meeting is an official activity of the Latin American Nutrition Society (SLAN) and its proceedings will be published as a special number of the SLAN journal, "Archivos Latinoamericanos de Nutrición".

J. E. Dutra de Oliveira, M.D.

Studies on the nutritive value of beans

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The potential use of food legumes as a source of protein to improve human nutrition has long been on the minds of people interested in food and nutrition.

Food legumes have on the average twice as much protein as cereals, and various species are consumed all over the world. It should be pointed out that the consumption of legumes ranges from insignificant amounts in Europe to intakes of 400 to 500 g daily in some parts of Asia and Africa (1).

In several countries in Central and South America food legumes, mainly common beans, is a daily component of the diet and average consumptions of 50 to 100 g are commonly found (2).

An extensive world review on the importance of legumes in human nutrition was published in 1964 by Aykroyd and Doughty (1). The importance of legumes as a source of energy, protein, and some minerals and vitamins is pointed out. The importance of processing and the presence of undesirable factors affecting the nutritive value of legumes seeds is also stressed.

The nutritive value of the protein content of leguminous seed was also reviewed in India by Venkat Rao et al. in 1964 (3). India was said to be the largest producer of legumes in the world. It had an availability of 60 g/caput/day of legumes and this would be equivalent to a daily supply of 12 to 15 g of protein. The authors call attention to the large variation in nutritive value of the legume seed proteins and to the fact that this food is not widely used in infant and small child feeding. In 1966, Oyenuga from Nigeria (4) reviewed the importance of the legumes in the improvement of the nutritional status in developing countries.

He points out to the amino acid compositon of different legumes, showing that the sulphur amino acid and tryptophan content of several of them it not as low as has been thought previously. The new provisional amino acid pattern published this year (5), decreasing the suggested levels of methionine, cystine and tryptophan, is certainly in accordance with this ideas.

In spite of this knowledge and of publications on the nutritive value of beans, their nutritional qualities and food use have not been fully understood or appreciated. The number of publications, meetings and applied programs dealing with food legumes has been relatively small.

Recently with the legumes meetings of the Protein Advisory Group held in Rome in 1972 (6), the International Center of Tropical Agriculture meeting in Cali, Colombia (7) and the present meeting in Brazil it seems that we are again trying to show the importance of food legumes in nutrition.

We have been interested in this subject for several years. In 1970 at a meeting in INCAP (8) on Protein Supply in Latin America we called for a better utilization of conventional foods as a source of protein. We tried to show the importance of conventional foods, such as rice and beans, as very abundant and easily available sources of dietary protein.

Our approach to the nutritional problem has also been to look upon it as a multidisciplinary subject. Our experiments have been dealing with aspects of agriculture, food technology as well as consumption and utilization of food. A report of some of our results is presented.

Influence of agricultural practice on the nutritive value of legume seeds

It has been shown, mainly in cereals, that nitrogen fertilization often increases the protein concentration of the grain. It is also true that an increase in the amount of protein does not mean an increase in the relative composition of the more limiting amino acids in the grain protein and often means a decrease (9).

Protein and yield is said to correlate negatively for soybeans, but this is not well known for other beans varieties. It has been also shown there is an inverse relation between protein and methionine content of beans (10). Omueti and Oyenuga (11) reported that in Nigeria raising soil phosphorus increases the amount of protein in groundnut and cowpea, but there is no report on the nutritive value of their proteins.

One of the work of our group was carried out to demonstrate the influence of N, K and P on the yield and nutritive value of common beans (12).

A factorial assay 3^3 was carried out using 0-40-80, 0-60-120 and 0-40-80 kg/ha of N, P, and K, respectively. A summary of the results is shown in Table 1, where an independent 4-fold increase in yield can be seen along with the corresponding protein content of the beans.

There was no correlation in yields and protein content. It is shown that fertilizer increased yield and total protein production per area.

A study of the nutritive value of these legumes was carried out in rats. The weight gain and protein efficiency ratios (PER) obtained in some of the rat assay samples are shown in Table 2.

These results seemed to us of importance because they showed in some cases a slight improvement of the biological value of the protein, but they also showed a decrease on the nutritive value in others. We do not know whether this was linked to the protein, mineral content, or increased synthesis of toxic substances with these treatments.

TABLE 1
YIELDS AND PECENT OF PROTEIN AS INFLUENCED BY FERTILIZERS

TREATMENT N P K	YIELD Kg/ha	PERCENT PROTEIN g
0 0 0	585	22.0
1 0 0	740	19.0
0 1 0	1760	23.3
0 0 1	633	19.7
1 2 0	2243	19.6
2 2 2	2283	20.8

TABLE 2
RAT NUTRITIVE VALUE OF COMMON BEANS AS INFLUENCED BY FERTILIZERS

TREATMENT N P K	WEIGHT GAIN g	PER
0 0 0	14,7	1.22
1 0 0	24.7	1.13
1 1 2	43.3	1.56
0 2 2	12.0	0.94
1 2 0	-8.8	--
1 1 0	-6.8	--

TABLE 3
NUTRITIVE VALUE OF SOYA BEANS AND FERTILIZATION

NITROGEN Kg/ha	PROTEIN CONTENT g	PER
0	43.7	0.47
25	41.9	0.97
50	44.6	1.19
75	41.4	1.50
100	43.1	1.43

Another study was carried out on fertilization and nutritive value of soybeans (13). Four different levels of nitrogen were applied in soybeans (25, 50, 75 and 100 kg/ha). The chemical composition of the beans were similar for protein but the nutritive value of the protein was shown to be higher on the samples that received fertilizers, as can be seen in table 3.

Nutritive value of common bean protein concentrates

The preparation of protein concentrates from legume seeds, mainly from soybeans, has been carried out by different processes and laboratories. Several washing processes will immobilize the protein and remove soluble sugars, minerals and other products.

This approach to the production of legume protein concentrates or isolates can be of importance in nutrition, because it has been shown that one of the problems with the utilization of the protein of legume food is the bulk, especially for infants and small children.

In our laboratory we extracted three bean fractions with a NaCl solution, HCl and heat (14). Total protein content of the fractions ranged from 59 to 66 percent.

Studies of the nutritive value of these fractions showed they were unable to support growth in rats. It was also shown that this failure was linked to the methionine deficiency of the product. Digestibility of the fractions was increased and hemaglutinin was destroyed by the treatment (Table 4).

Methionine infused common beans

The methionine deficiency of common beans is well known. Addition of methionine greatly increases the nutritive value. A methionine-infused common bean would open the possibility of utilizing a certain number of treated beans in addition to normal beans in order to reach desirable supplementary levels in the mixture.

We approached this problem studying the infusion of methionine in beans subjected to different solutions and temperatures. Precooked beans with absorbed methionine were obtained. The quantity of this amino acid per 100 g of our product is shown in Table 5 and in Table 6 is shown the nutritive value of different samples (15).

TABLE 4
NUTRITIVE VALUE OF PROTEIN FRACTIONS ISOLATED FROM *Phaseolus vulgaris* IN RATS

	PER	DIGESTIBILITY %
COOKED BEANS	1.16	73
COOKED BEANS AND M	2.48	74
FRACTION A AND M	2.30	89
FRACTION B AND M	1.99	82
FRACTIONS A AND B AND M	2.21	83
CASEIN	3.00	92

* Methionine.

TABLE 5
COMMON BEANS METHIONINE ABSORPTION

PROCESS	METHIONINE SOLUTION CONCENTRATION	METHIONINE BEANS mg/g N
SOAKING	3.5	577
	2.0	407
	0.5	233
COOKING	3.5	1375
	2.0	1300
	0.5	449

TABLE 6
NUTRITIVE VALUE OF METHIONINE INFUSED COMMON BEANS

EXPERIMENT	METHIONINE BEANS mg/g N	PER
	1375	1.27
A	1300	1.82
	449	2.89
	1291	1.25
B	674	1.25
	378	2.45

One of the problems with this approach and one which has been a big challenge to the food technologist is that of having good-looking precooked processed beans. On the other hand, with the need for more convenience foods I see the possibility of utilizing this process. For beans, this process has a further advantage because both the beans and the water in which they are cooked are consumed, and as such there is no danger of loosing the added amino acid.

Effect of germination and soaking on the nutritive value of beans

It has been known for a long time that germinated or sprouted legumes would prevent scurvy. This was traced and proved to be linked to synthesis of vitamin C by the legume seed during germination. Changes in other nutrients, such as niacin and carbohydrates, are also known to occur with germination. We carried out a study of the effect of germination of common beans *(Phaseolus vulgaris)* on the nutritive value of its protein (16).

Samples of common beans were left germinating for 2, 4, 8 and 10 days. The germinated samples were dried, autoclaved and assayed in rats for nutritive value. A decrease of the nutritive value of the protein from common beans during germination was found.

Soaking of beans before cooking has been a standard procedure in our country. We also investigated the effect of different soaking times on the nutritive value of bean protein and the results showed a decrease in the nutritive value with prolonged soaking time (Table 7). This can be of practical importance since overnight soaking is common because, among other things, it decreases cooking time (17).

TABLE 7
SOAKING TIME AND NUTRITIVE VALUE OF BEANS

TIME	WEIGHT GAIN g	PER
30 min.	16	1.00
6 hrs	14	0.91
12 hrs	8	0.66
18 hrs	9	0.70
24 hrs	9	0.66

Nutritive value of home cooked and autoclaved soybeans

Our group has been carrying out several research projects dealing with the nutritive value of soy products. Several years ago we tested the nutritive value of different defatted soy flours manufactured in Brazil and compared them with similar products from the United States and with casein. The results showed that they have a lower nutritive value than casein but that this value can be increased by the addition of methionine (18). Our results also show that the full fat soy flour has a better nutritive value than defatted soy flour (18). Isolated soy proteins manufactured both in our country and abroad have also been tested in our laboratories and they also have, in rats, a lower nutritive value than casein, improved by the addition of methionine. Studies with normal and malnourished children in our metabolic unit were carried out feeding them several soy products. Different soy products have a fairly good nutritive value. Soy milk fed at 3% protein level to malnourished children had a nutritive value similar

to cow's milk (19). The importance of methionine as a supplement to soy products (milk and isolated) has also been the subject of metabolic studies in our laboratories (20).

I am not going to discuss all these experiments but I would like to show some results of another study on the influence of home cooking and autoclaving on the nutritive value of soybeans (21). It is generally accepted that soybeans and other legume foods are methionine deficient when rat tested. Our results, shown in Table 8, seem to demonstrate the importance of cooking time on their nutritive value. It seems highest nutritive value, similar to caein, can be brought out by specific heat conditions. This was mainly achieved in our experiments by both autoclaving (Table 8) and home cooking (Table 9). It also appears that a decrease of the nutritive value of the soya can also be a result of excessive heating.

TABLE 8
NUTRITIVE VALUE OF AUTOCLAVED SOYA BEANS

TIME MINUTES	PER
	0.77
0	2.85
5	2.75
10	2.67
20	2.54
30	2.39
60	2.13
CASEIN	2.94

* Heated to autoclaving temperature.

These aspects seem of great importance from the practical point of view, because it seems that excessive concern with toxic factors in soy and other legume foods is a current idea and can be responsible for a reduction of their protein value.

Common beans as a food mixture in Brazil

Common beans are a staple and daily food all over Brazil. They are eaten in the Northeast with cassava flour and in the South with rice.

TABLE 9
NUTRITIVE VALUE OF HOME COOKED SOYA BEANS

TIME HOURS	PER
0	0.77
2	2.47
3	2.11
4	3.06
5	2.22
CASEIN	2.94

TABLE 10
NITROGEN RETENTION OF PRE SCHOOL CHILDREN FED A BEAN AND RICE DIET

BEANS AND RICE N RETENTION %	BEANS + RICE + MILK N. RETENTION %
6.7	8.7
11.2	16.8
11.3	17.6
17.6	23.8
22.6	31.4

We studied the nutritive value of a rice and bean mixture. In rats we showed that the best combination of their protein is 85% from rice and 15% from beans (22). Preschool children fed a rice and bean diet similar to the ones normally fed them by mothers of low socioeconomic level (200 g rice and 56 g of beans) retained less nitrogen than when part of the diet was replaced by milk (23) as shown in Table 10.

Further studies have shown that the nitrogen retention of the rice and bean diet can also be improved through the utilization of a corn-soya mixture (Table 11), reaching higher levels than those found with milk (23).

TABLE 11
NITROGEN ABSORPTION AND RETENTION OF PRE SCHOOL CHILDREN FED RICE AND BEAN DIETS

DIET	ABSORPTION %	RETENTION %
RICE + BEANS	56.4	13.9
RICE+BEANS+MILK	71.1	19.7
RICE+BEANS+CORN + SOYA	69.4	25.5

These studies point out that a balanced and well known food, such as cow's milk, is not always the best supplement to a local diet. A better supplement for the rice and bean diet seems to be this corn-soy mixture rather than cow's milk.

Considering that in the Northeast of Brazil cassava flour is eaten together with beans we have been advising the utilization of the flour as a carrier of nutrients. In our especific situation we did studies (24, 25) on the use of cassava flour as a methionine carrier to balance common bean-based diets. Experimental results show a striking improvement of the nutritive value of these diets, when methionine is present in the flour (Table 12).

TABLE 12

INFLUENCE OF METHIONINE ON COMMON BEAN BASED DIETS

PROPORTION MANIOC TO BEANS	PER	METHIONINE
65:35	0.78	NO
	2.70	YES
55:45	0.97	NO
	2.68	YES
45:55	1.28	NO
	2.27	YES

SUMMARY

Studies on the influence of different factors on the nutritive value of legume seeds showed:

1. The use of fertilizers will greatly increase the yield of common beans, but their influence on the protein content of the beans varies. It was shown that fertilization exerts an influence on the nutritive value of the seed protein.
2. Common bean protein concentrate obtained in our laboratory was unable to maintain growth in rats. Methionine deficiency seemed to be the main factor, considering that digestibility was improved and toxic factors were low.
3. When common beans are infused in a methionine solution this amino acid is increased and can be used as a suplement to normal precooked beans.
4. Soaking and germination of common beans will not increase, but can decrease, their protein nutritive value.
4. Time of home cooking and autoclaving will change the nutritive value of soybeans.
6. Metabolic studies with preschool children have shown that a rice-and-bean local diet can be improved by replacing part of it with cow's milk or a corn-soy mixture.
7. Experimental studies in rats have also shown that cassava-bean diets greatly improved their nutritive value with methionine. It was suggested and shown how to utilize cassava flour as a methionine carrier to balance such diets.

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Utilization of legumes in poultry feeding

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The legumes are important and economical sources of protein nd energy in human nutrition, some are used for animal feeding. 'he protein of the legumes is deficient in methionine and cystine nd some contain trypsin inhibitors and other toxic principles. ;ven thought legumes are important food crops in many parts f the world there is not enough published information on its utrient content. In the case of *Cicer arietinum* (garbanzo), Russell t al. (18), observed that the addition of 0.1% methionine to 10% protein garbanzo diet for rats gave an increase in weight ain.

Jaffé, (13), reported that garbanzo autoclaved prior to mixing ı the diet supported rat growth comparable to that obtained ;ith soybeans and that methionine addition gave a marked impro- 'ement in nutritional value. Cuca et al (7) reported that as garbanzo ncreased in the diet, weight of poults and feed efficiency decreased. Autoclaved garbanzo improved poult weights significantly, methio- ıine supplementation to raw garbanzo diets increased poult weights. Cuca and Pró (9) made a review of this subject.

Broad beans *(Vicia faba)* according with Mahon and Common 15) are deficients in methionine and cystine, and deficient to ı lesser degree in tryptophan, glicine, plenylalanine, and threonine. Borchers and Ackerson (4) found no trypsin-inhibitor activity; ıowever in (5) they reported that autoclaving improved the ıutritive value of broad beans as measured by rat growth. Bletner *:t al* (3) indicated that broad bean seeds may be used as a source ɔf vegetable protein for chicks during the first four weeks of ıge, when the diets are supplemented with other source of ɔrotein.

Kienholz *et al* (14) found that autoclaving peas *(Pisum sativum)* greately increased the nutritional value for young chicks even though according to Bochers *et al* (5) trypsin inhibitor was not present. Beans *(Phaseolus vulgaris)* are used primarely as a source of protein for humans, however according with Fernández *et al* (11) in situations of surplus or unsuitability of the beans for human consumption, they may become useful as feed ingredient for poultry. In this paper several experiments to study the value of some legumes in diets for poultry are reported.

Experimental procedures
Chick garbanzo experiments

Garbanzo used was ground in a hammer mill through a one-eight inch screen. In treatments where the garbanzo was autoclaved, it was placed in an autoclave for thirty minutes at ten pounds of pressure, dried at room temperature, reground and then added to the diets.

Day old White Olympian or White Leghorn chicks were used as experimental animals. The birds were randomly distributed into pens of ten each. Three replicate groups were fed each diet. The chicks were maintained in electrically heated batteries with raised wire floors. Feed consumption and body weights were measured each week of the experiment. Water and feed were supplied *ad libitum* and continuous light was provided. Composition of diets are given in Table 1.

Experiment 1

In this experiment garbanzo was autoclaved in a steam atmosphere with and without added water. The methods were as previously described. Day-old, staight-run White Olympian chicks were used.

Results and Discussion

Average weights and feed efficiency are shown in Table 2. The results showed a statistically significant ($P > 0.01$) growth response to autoclaving garbanzo. No differences were observed between the two methods of autoclaving, also no response was observed when the autoclaved garbanzo was supplemented with fat. The feed efficiency was improved for autoclaved garbanzo. Addition of fat to raw garbanzo slightly improved growth and feed efficiency.

TABLE 1

COMPOSITION OF EXPERIMENTAL DIETS (EXPERIMENT 1 AND 2)

Ingredients	Exp. 1	Exp. 2
	Percentage	
Garbanzo	76.00	80.00
Soybean Oil Meal (50% protein)	- - -	4.77
Isolated soybean protein (C-1 assay)	3.50	- - -
Cerelose	4.97	- - -
Tallow	2.00	2.00
Fish Meal, herring (73% protein)	5.00	5.00
Dehydrated alfalfa meal (17% protein)	3.00	3.00
Limestone	2.50	2.50
Dicalcium phosphate	1.50	1.50
Iodized Salt	0.50	0.50
Potassium (KCL)	0.20	- - -
Manganese ($MnSO_4$)	0.03	0.03
Zinc ($ZnSO_4$)		0.013
Methionine (MHA)	0.30	- - -
Vitamin Mixture[a]	0.50	0.68

[a] Vitamin mixture supplied the following ingredients per kg: Vitamin A 5280 U.S.P.; Vitamin D_3 396 I.C.U.; Thiamine Hcl, 3.5 mg; - Riboflavin, 6.29 mg; Pantothenic acid, 20.24 mg.; Niacin, 58.08 mg.; Pyridoxine, Hcl, 6.29 mg., Choline Choloride, 1936 mg.; and Bacitracin, 11 mg.

TABLE 2

EFFECT OF AUTOCLAVING GARBANZO IN A STEAM ATMOSPHERE WITH AND WITHOUT ADDED WATER ON CHICK GROWTH AND FEED EFFICIENCY

Treatment of Garbanzo or Supplement	Average Weight Twenty-Six Days g	Feed Efficiency
1. None	263[a]	2.20
2. 5% Tallow	287[a]	2.18
3. Garbanzo autoclaved dry	328[b]	2.02
4. Garbanzo autoclaved wet	322[b]	1.96
5. Garbanzo autoclaved dry + 5% Tallow	338[b]	1.93
6. Garbanzo autoclaved wet + 5% Tallow	323[b]	1.91

Numbers with different letter are significantly different ($P<0.01$)

In this experiment, more than 15% of the chicks were affected with perosis, suggesting a manganese or zinc deficiency, even though it was thought that the diets contained sufficient amounts of both elements. It could be that garbanzo in some way or another increased the requirements for these minerals.

O'Dell and Savage, (16), demonstrated a difference in zinc requirements of chicks fed casein or isolated soybean protein (C-1) assay diets and concluded that this isolated soybeans protein made zinc unvailable to the chicks. In the present experiment it is possible that the zinc in garbanzo-isolated soybean protein diet was unavailable to the chicks. However, only 3.5% isolated soybean protein was used, compared to 15% used by O'Dell and Savage (16).

The results of this experiment with chicks agree with those of Jaffé (13) and Hirwe *et al*, (12), who found that garbanzo was improved for rats by autoclaving.

Experiment 2

The object of this experiment was to determine the influence of raw and autoclaved garbanzo an methionine requirements of chicks and to determine the influence of supplemental methionine as well as autoclaving garbanzo on the pancreas size of chicks. Day old, straight-run, White Olympian chicks were used.

Results and Discussion

Average weights and feed efficiency are in Table 3. At the end of the experiment nine chicks fed diets 1, 3, 7 and 9 were sacrificed by dislocation of the cervical vertebra. The entire pancreas was removed from each and weighed. Average pancreas weights are in Table 4.

Raw garbanzo was significantly improved by addition of methionine. The growth of chicks fed methionine supplemented garbanzo approached that of chicks fed autoclaved garbanzo. When methionine was added to the basal diet at levels of 0.15 0.3, 0.45, 0.6, and 0.75%, a similar growth response was obtained with all levels. Therefore, the 0.15% level apparently was adequate. In

TABLE 3

THE EFFECT OF GRADED LEVELS OF METHIONINE SUPPLEMENTATION TO RAW AND AUTOCLAVED GARBANZO ON CHICK GROWTH AND FEED EFFICIENCY

Diet No.	Treatment of Garbanzo	Percentage Methionine (MHA) added	Average Weight four weeks g	Feed Efficiency
1		none	291[a]	2.17
2		0.15	315[abc]	2.14
3	none	0.30	320[abc]	1.96
4		0.45	301[ab]	2.12
5		0.60	325[bc]	1.97
6		0.75	316[abc]	2.03
7		none	330[bc]	2.03
8		0.15	330[bc]	2.01
9	autoclaved	0.30	336[c]	2.09
10		0.45	329[bc]	1.98
11		0.60	332[bc]	1.99
12		0.75	343[c]	2.00

TABLE 4

EFFECT OF AUTOCLAVING GARBANZO AND OF SUPPLEMENTAL METHIONINE ON PANCREAS WEIGHT

Diet	Average Chick Weight g	Average Pancreas Weight	M. Pancreas/ 100 gm. body Weight
1 Raw Garbanzo	311	1920	617.36
7 Autoclaved Garbanzo	351	1370	390.31
3 Raw Garbanzo + 0.3% Methionine	310	1040	529.03
9 Autoclaved Garbanzo + 0.3% Methionine	334	1220	365.27

contrast autoclaved garbanzo was not improved by addition of methionine. This suggests that the improvement of garbanzo by autoclaving was through increasing availability of methionine, since this aminoacid added to untreated garbanzo was almost as effective as improving growth as the combination of autoclaving plus methionine.

A pronounced difference in pancreas weights was evident between raw and autoclaved garbanzo. Addition of methionine did not affect pancreas weight. These results are similar to those of Chernick *et al.*, (10) with chicks fed raw and autoclaved soybean oil meal. The difference in pancreas weight of birds fed raw and autoclaved garbanzo may be due to absence or unavailabitity of some substance(s) or to the presence of a heat labile inhibitor(s) in raw garbanzo that causes hyperplasia (2) of the pancreas.

In this experiment a low incidence of perosis was observed in contrast to the higher incidence observed in previous experiment. It is possible that the lower incidence in the present experiment was due to the substitution of soybean oil meal for isolated soybean protein.

Garbanzo in the diets for laying hens
Experimental procedure
Experiment 3

In this experiment, garbanzo was used as the principal source of protein and energy, the composition of the experimental diets is shown in table 5. It can be seen that in diet number 1, 15% milo was used as an additional source of energy, and in diet 2, 6.5% molasses was used for energy supplementation. Diet 3 was used as a control diet. In this experiment, 153 pullets were distributed at random in three replicate groups with 17 birds per group, 51 per treatment. The birds were kept in individual laying cages and the experiment started when they were approximately six months of age and was continued for 26 weeks. Water and feed were supplied *ad libitum*. Artificial light was provided from 5 A.M. to daylight throughout the trial. Feed consumption was recorded weekly and egg production on a daily basis.

Experiment 4

In this trial, a factorial experiment was conducted which included two levels of protein (14.75 and 16.5%) in four combinations with blood meal (0, 1, 2 and 3%). The composition of the diets may be seen in Table 6. The only amino acid sup-

plementation was with methionine in order to maintain a level of 0.53% in all diets. Leghorn pullets six months of age were placed in individual laying cages. They were observed during a 2 weeks per treatment period so as to eliminate non-layers. Three replicate groups of ten birds each were assigned to each treatment. Water and feed were supplied *ad libitum*. Artificial light was provided from 5 A.M. to daylight. Egg production was recorded daily while feed consumption was determined weekly for each repetition. The duration of the experiment was 17 weeks.

TABLE 5
COMPOSITION OF DIETS (APP. 15% PROT.) USED IN EXPERIMENT 3 ON EVALUATION OF GARBANZO FOR LAYING HENS (SANTA ELENA)

Ingredient	Treatment No.		
	1	2	3
Garbanzo (chick peas)	74.0	83.0	-
Milo	15.5	-	-
Corn	-	-	57.2
Sugar	-	-	14.9
Fish Meal (70%)	-	-	1.3
Meat Meal (50%)	-	-	1.7
Soybean oil meal (50%)	-	-	13.8
Alfalfa meal	3.0	3.0	4.2
Molasses	-	6.5	-
Oyster shell	3.2	3.2	3.1
Phosphate rock	4.0	4.0	3.4
Salt	0.3	0.3	0.3
Trace mineral mix (ccc)	0.05	0.05	0.03
Vit. A (20, 000 I.U./g.)	20 g.	20 g.	16.5 g.
Vit. D.(20, 000 I.U./g.)	44 g.	44 g.	35.0 g.
Riboflavin	0.44 g.	0.44 g.	0.15 g.
Calcium Pantothenate	-	-	0.40 g.
Choline Choloride	-	-	66.00 g.
Niacin	-	-	0.40 g.
B_{12} (44 mg./ Kg.)	1.1 mg.	1.1 mg.	1.95 g.
Calc. Prot.	15.5	15.6	15.04

Results

Experiment 3

The results of this trials are summarized in table 7. It can be seen that when garbanzo was used as the principal nutrient source, egg production declined very rapidly in both garbanzo diets as compared with the corn soy diet. Interestingly enough the decline in egg production was less in diet 2 containing the high level of

garbanzo. At the end of the third week and because of the decline in egg production of the birds on the garbanzo diets, it was decided to add 2% blood meal to diets 1 and 2 as an amino acid supplement.

TABLE 6
COMPOSITION OF BASAL DIETS WITH DIFFERENT LEVELS OF BLOOD MEAL

Ingredient %	Diets 14.75% protein	16. % protein
Garbanzo	79.09	86.53
Alfalfa	2.67	3.00
Blood meal	0.00	0.00
Sugar	7.72	0.00
Premix*	10.34	10.34
Methionine	0.18	0.13

* Added in percentage: Oyster shell, 3.70; salt, 0.3; vitamins and minerals 0.34, sesame oil 2.0

Three weeks after the addition of blood meal, egg production improved and from the seventh to the 26 week, egg production was almost equal to that of the hens on the control diet. It may be noted that the average daily feed consumption was higher in the garbanzo diets, which has been a consistent observation in previous studies, Pino *et al* (17).

Experiment 4

The results of the this experiment at the end of 17 weeks did not show any significant difference among treatments in regard to egg production and feed consumption. These are shown in Table 8. These results are in contrast with those of the experiment in which garbanzo, when used as the principle source of energy and protein caused a rapid decline in egg production. In this experiment, good egg production was obtained throughout the treatment period. The addition of blood meal at each dietary protein level did not influence egg production as was observed in the previous experiment. Of interest also is the observation that the diets containing 14.75% protein supported as good egg production

as those with higher protein levels, indicating that the nutrient requirements were met on this simple ration perhaps due to the high feed consumption. It was further observed that body weight at the and of the experimental period showed a similar increase in all diets.

TABLE 7
EGG PRODUCTION OF HENS FED CHICK PEAS AS THE MAIN SOURCE OF PROTEIN (SANTA ELENA)

Weeks on Exp. diet	Treatment 1	2	control
1	76.5	69.2	46.6
2	42.6	58.7	55.5
3	37.4	53.0	72.3
		Addition of Blood Meal	
		Recovery Period	
4	39.7	56.1	74.0
5	46.0	58.6	80.2
6	59.4	62.6	77.4
		Post Recovery	
7-26	66.4	66.2	69.5
	Average Feed Comsuption g/ day/ bird		
	130	143	120

No satisfactory answer can be given for this difference in both experiments, since the hens were more or less of the same age, althought of different commercial source or perhaps the garbanzo samples were different. At this point it is important to mention the results of Aguilera *et al* (1). These authors reported that the garbanzo lysine is as available as the synthetic one. Cuca and López (8) also inform that the lysine is available in 94% for chicks.

Experiment 5

In this experiment the legumes were autoclaved at 15 pounds of pressure, the method used was similar to the garbanzo experi-

ment. Seven day old male white Leghorn chicks were used. The birds were randomly distributed into pens of eight each. Three replicate groups were fed each diet (the composition of the experimental diets are in table 9). The protein content was calculated to be 15.5%; most of the protein was provided by the legume, the rest came from the milo, oil was added to increase the energy. All diets were supplemented with 0.3% D-L methionine. Feed consumption and body weights were recorded each week of the experiment

TABLE 8
EGG PRODUCTION OF HENS FED GARBANZO SULEMENTE WITH BLOOD MEAL

Treatment		Results 17 weeks	
		% egg production	g feed/egg
1		74.4	184
2	14.75% protein	73.4	177
3		70.6	182
4		71.7	182
5		72.1	189
6	16.5% protein	70.7	180
7		68.6	186
8		74.6	178
Control diet		71.8	159

TABLE 9
COMPOSITION OF DIETS USED TO STUDY THE NUTRITIVE VALUE OF SOME LEGUMES

Ingredient	Treatments				
	1 Clitoria	2 lentils	3 broad beans	4 beans N-66	5 beans F. mayo
Milo	63.915	38.950	41.635	26.765	11.350
Legume	28.100	53.065	50.380	65.250	80.665
Vit. and minerals[1]	4.985	4.985	4.985	4.985	4.985
Oil	3.000	3.000	3.000	3.000	3.000
Methionine	+	+	+	+	+
Calculated protein	15.48	15.49	15.49	15.48	15.50

1/ In percentage: Bone meal, 4.0, K H_2 PO_4, 0.56; Nacl, 0.3; Mn SO_4. H_2O, 0.0225; Zn CO_3, 0.00065; $CaCO_3$, 0.0016; KI, 0.00025.

Supplied per kilogram of diet: Vit A, 8000 I.U.; Vita D_3, 1250 I.C.U.; Vit E, 5, U.I.; Riboflavin, 4 mg.; D-Pantothenic acid, 15 mg; Niacin, 25 mg.; Choline chloride, 200 mg.; Vit. B_{12}, 0.015 mg.

Results

The results are in table 10. In the case of *Clitoria ternata* (CT) it can be seen that the chicks fed raw (CT) lost weight, and when this legume was autoclaved a significant increase in body weight was observed althought the gain is 6 time less as compared with the control diet; this indicates that probably it is necessary to study more this legume, and supplement it with some other amino acids since apparently (CT) is deficient in several amino acids, or perhaps, it contains some toxic substances that are not destroyed by heat as suggested by Bravo (6) who indicates that compounds related to tannic acid (4.6% as quercitanic acid) might be responsible for the depression in rate gain of rats. The weights of chicks fed raw lentils were less as compared with the control diets. The weights significantly improved when autoclaved, although they were not similar to the control diet. Perhaps as in the case of (CT) it is necessary to supplement some other amino acids. Raw Broad Beans *(Vicia faba)* gave weights almost similar to the control diet, however, when autoclaved a significant increase in body weight was observed. This results are in agreement with those of Borchers and Ackerson (5) in rats; it is important to mention that the same authors in (4) found no trypsin inhibitor in broad beans. Bletner *et al* (3) indicated that when 33% raw broad beans were used, chick growth was unsatis-

factory. These results are similar to the ones observed in this experiment where broad beans were used in raw form, but not when autoclaved.

Two varieties of common beans were fed raw and autoclaved. One was the Negro 66, a tropical bean, and the other a high altitude variety. The results indicate a loss of weight and a 41.66% mortality, this results are different from the ones of Fernández and McGinnis (11) although they are similar when the beans were autoclaved. The other variety used was flor de mayo, with this beans, the chickens lost weight but it was less as compared with the tropical beans, the response was less when autoclaved. Probably there is a great variation among varieties in their composition that produced these results.

TABLE 10

EFFECT OF AUTOCLAVING SOME LEGUMES ON CHICK GROWTH AND MORTALITY

	Treatment	Results 14 days gain %	mortality %	
1	Raw clitoria	-3.43	8.3	(2)
2	Autoclaved clitoria	8.64	8.3	(2)
3	Raw lentils	23.04	0.0	(0)
4	Autoclaved lentils	40.24	4.1	(1)
5	Raw broad beans	45.95	4.1	(1)
6	Autoclaved broad beans	72.10	0.0	(0)
7	Raw beans N-66	-14.03	41.66	(10)
8	Autoclaved N-66	22.34	25.0	(6)
9	Raw beans flor de mayo	- 8.13	83.0	(15)
10	Autoclaved flor de mayo	7.00	41.6	(10)
11	Control	51.32	8.3	(2)

SUMMARY

A series of experiments were conducted to study the nutritive value of garbanzo, beans, broad beans, lentils, and Clitora ternata for poultry. When garbanzo was used in diets for chick a significant growth response was observed when autoclaved. Raw garbanzo was significantly improved by the addition of methionine (0.15%), a pronounced difference in pancreas weight was evident with raw and autoclaved garbanzo. In diets for laying hens the results indicated that when used as the main source of energy at 15% protein level, egg production declined very rapidly, but when supplemented with 2% meal, egg production was almost equal to that of the hens in the control diet. In diets with 16.5% protein garbanzo gave similar egg production to the control diet. Broad beans (Vicia faba), when used in combination with milo in 15.5% protein diets a significant improvement in body weight was observed when autoclaved. Lentils were improved by autoclaving but broad beans has a better improvement. The two varieties of common beans responded to autoclaving but the tropical variety responded better. A high mortality was observed in both beans, raw or autoclaved and supplemented with methionine, although it was higher in the raw beans. Clitoria ternata responded to autoclaving, however, the chick growth was very poor as compared with the control diets.

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Utilization of legumes seeds in swine feeding

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Under modern production conditions, pigs need to be fed diets containing 75 - 85% of energy-providing feeds such as cereal grains and 15 - 25% of good quality protein sources such as oil-seed, animal, or marine by-products.

The main pork-producing countries are also the main cereal grain producers, so after meeting the human needs for cereals, grains are then available for use as animal feeds; the sources of protein used for swine feeding in such countries are by-products not used as human foods. In the case of developing countries, the insufficient food producing capabilities make swine and other non-ruminant animals direct competitors of man for the small food supplies available. In such countries, swine should be fed agricultural wastes and industrial by-products that could not be used for human consumption, such as the by-products of the sugar cane and the starch industries as sources of energy, and the by-products derived from the food packing and processing industries, the damaged agricultural products and the crystalline amino acids as sources of protein. Cull legume seeds are among the agricultural products that could be used as swine feeds.

The published information regarding the use of most legume seeds for swine feeding is scarce and incomplete. The only possible exceptions are soybeans and peanuts, which in the form of oil-meals have been extensively studied throughout the years. The present review will not include such by-products, but will concentrate in whole seeds.

Peas *(Pisum sativum, P. arvense).*

The peas that are used in swine feeding are known as cull peas and consist of split, small and damaged seeds (1).

When cull peas were used as a protein source for fattening hogs and were combined with meat meal in wheat diets, pigs performed

better than animals fed pea-wheat diets. The former diet contained 57% peas and 10% meat meal (2).

When four combinations of wheat and peas were offered *ad libitum* with a protein supplement, pigs on wheat-pea diets gained more than those fed wheat alone. The best grain combination was observed to be 80% wheat and 20% peas. When peas accounted for half of the grain mixture, the diet was found to be unpalatable (3).

Two wheat-pea combinations were supplemented with either meat or pea meal and it was observed that the 60 : 35% diet performed better than the 75-20% combination with either protein supplement. At both grain levels, meat meal was a better source of supplementary protein than pea meal (4).

Cull peas were compared with meat meal and soybean meal as sources of protein in wheat-barley rations. Peas and meat meal resulted in significantly better daily gains than soybean meal. The best feed/gain ratio was obtained with the pea diet. In a second experiment, cull peas resulted in a significantly better performance than meat meal (5).

Ground Alaska field peas were compared with fish meal in growing and finishing pig rations. Performance of pigs to 90 kg indicated only a slight improvement in feed/gain ratio when fish meal was used. The addition of chlortetracycline improved both diets (6).

A diet containing 65% peas *(P. arvense)* was compared with one containing 40% peas and 10% meat meal. The average daily gains and feed/gain ratios were 799 g, 2.24; 709 g, 4.29, respectively (7).

Several combinations of field peas, either raw or cooked, combined with a fish-meat meal mixture were found to result in only small differences in growth, feed conversion and dressing percentage between diets. Results of feeding rations containing 20% of either raw or cooked field peas and 12% of the fish-meat meal mix showed that there was not a beneficial effect of cooking. Total gains for the raw and cooked pea diets were 32.5 and 32.4 kg; feed/gain ratios were 3.75 and 3.78; dressing percentages were 76.7 and 77.0%, respectively (8). When peas were compared with fish meal at different dietary combinations it was observed that fish diets resulted in greater gains than all-vegetable diets (9).

The effect of preparation of peas on their nutritive value for five-month-old pigs was studied (10). Animals were fed 90-days

with diets consisting in 43% maize, 11% barley, 2.5% alfalfa meal, 18.5% sugar beets and 25% peas. The latter were in the following forms: 1) meal; 2) boiled at pH 8.4 for one hour; 3) germinated and fermented for 2 days; 4) meal + lysine + methionine; 5) meal + vitamin B_{12} + biomycin; 6) control of meat and bone meal (6%), yeast (7%) and starch (12%). Average daily gains were: 503, 556, 541, 527, 571 and 550 g, respectively. Feed/gain ratios followed the same pattern.

TABLE 1
FIELD PEAS (PISUM SATIVUM) VS. SOY-FISH MEAL FOR SWINE

Peas, %	.0	6.0	12.0	18.0	24.0
Soy-fish meal %	7.0	5.3	3.5	1.8	.0
Daily gain, kg	0.703	0.702	0.696	0.684	0.704
Feed/gain	3.07	2.95	3.35	3.26	3.06

Bell & Wilson (11).

In a recent work, field peas gradually replaced soy-fish meal with no significant effect on performance (Table 1). Also, the addition of methionine to a diet containing 10% field peas had no effect. In a second study, cull peas were substituted for barley at 10, 20, 30 and 40% levels. Diets were not isoproteic so the ones with high levels of peas provided protein in excess of the requirement. Performance tended to decrease with the increasing levels of field peas. Average daily gains were 806, 788, 752 and 679 g; feed/gain ratios were 2.91, 2,86, 3.15 and 3.29, respectively (11).

TABLE 2
PEAS (PISUM SATIVUM & PISUM ARVENSE) FOR SWINE

Raw vs. Basal	Heat Treatment	Methionine Supplementation	Reference
Worse	-----	-----	Lehrer & Hodgson (1946).
Better	-----	-----	Cunha et al. (1948).
Equal	-----	-----	Keith et al. (1962).
Worse	-----	-----	Lerner & Nardiello (1963).
Worse	no effect	-----	Lobban (1963).
Worse	-----	-----	Preš & Fritz (1966).
Worse	improved	-----	Šarov (1965).
Equal	-----	no effect	Bell & Wilson (1970).

Based on experimental results it is recommended that gestation-lactation diets include 20 - 30% peas (12).

The main results on the feeding value of peas for swine are summarized (Table 2).

Cowpeas *(Vigna sinensis)*.

Cull black-eyed cowpeas were included as a protein source in barley diets (13). The levels used were 20%, 20% + SOM + CSM, 50%. Animals on the latter diet gained more than those in the other diets; average daily gains were 718, 591, 563, 900 g, respectively. However, a wastage problem in the high cowpea ration was reflected on feed conversion: 3.65, 3.52, 3.65, 4.17, respectively. When cowpeas were fed alone for 28 days, daily gain decreased considerably (268 g).

Treatment of cowpeas with heat (toasting at 80 C) significantly improved total gain (2.53 *vs.* 4.13 kg), feed/gain (3.89 *vs.* 2.02) and slightly improved apparent protein digestibility (64 *vs.* 73%) for weanling pigs (14).

An experiment was conducted to determine the effect of germination and cooking on the nutritive value of cowpeas for growing swine, when the legume seeds supplied all the dietary protein (Table 3). Cowpeas were administered raw, germinated or cooked, with and without methionine. Germination failed to support growth even at the rate obtained with the raw unprocessed cowpeas. The addition of methionine to raw cowpeas had no effect on daily gain (551 *vs.* 483 g); however, there was a significant improvement on the feed/gain ratio with the addition of methionine (3.42 *vs.*

TABLE 3
EFFECT OF GERMINATION AND COOKING OF COWPEAS (VIGNA SINENSIS) FOR SWINE

	Corn-Soy	Raw	Germinated	Cooked
Dayly gain, kg	0.799^{a}	0.551^{bc}	0.498^{c}	0.816^{a}
Feed/gain	2.53^{a}	3.42^{b}	3.42^{b}	2.50^{a}

abc Values in a row with the same superscript do not differ significantly ($P<.05$)

CIAT (15).

TABLE 4
COWPEAS (VIGNA SINENSIS) FOR SWINE

Raw vs. Basal	Heat treatment	Methionine Supplementation	Reference
Better	-----	-----	Heitman & Howart (1960).
Worse	Improved	-----	Sevilla-Eusebio et al.(1968).
Worse	Improved	no effect	CIAT (1971).

3.09). When the sulphur amino acid was added to the germinated cowpeas, both performance parameters were significantly improved (498 *vs.* 649 g; 3.42 *vs.* 2.70) which seemed to indicate that germination had a detrimental effect on the sulphur amino acids. Cooking of the seeds resulted in growth and feed/gain ratios comparable to the ones obtained with the corn-soy control. The addition of methionine to the latter had no significant effect (15).

Studies on the feeding value of cowpeas for swine are summarized in table 4.

Horse bean *(Vicia faba)*.

The effect of including graded levels (0 to 30%) of horse beans in swine rations (55 - 90 kg) was studied (16). No significant difference in performance was observed when 10, 20 and 30% horse beans were used. However, pigs fed 0% horse beans gained better and more efficiently than those given 30%. The digestibility of the protein of the horse beans was 80% at all three levels but with 30%, the digestibility of the organic matter was reduced compared to the 20%. The energy of the digested feed decreased as horse beans were increased. When methionine was

TABLE 5
HORSE BEANS (VICIA FABA) AND SOYBEAN MEAL IN SWINE DIETS

	Horse beans	Horse beans + methionine	Soybean meal
Daily gain, kg	0.596[a]	0.734[b]	0.718[b]
Feed/gain	2.86	2.82	2.82

ab Values in a row with the same superscript do not differ significantly ($P<.05$).
Balboa et al. (18).

added to growing swine (18 to 55 kg) rations, there was an improvement in the efficiency of feed conversion.

Horse bean protein has been reported to be comparable to that of a combination of dried torula yeast and oilmeals (7).

In an experiment in which a corn-fish meal ration was supplemented with horse bean meal, horse bean meal + methionine, or soybean meal, there was a 140 g improvement in gain with the addition of methionine, and the difference between the latter treatment and the soy control was unsignificant. All three diets resulted in similar feed/gain ratios (Table 5) (18).

A series of experiments was conducted to study horse beans as a feed for bacon pigs (19). In a first trial, the gradual replacement of a soybean-meat-and-bone meal mixture by bean meal, with and without methionine, was studied and it was observed that when horse beans completely replaced the protein mix, the performance was the lowest. There was an improvement of all diets with the addition of methionine. In a similar experiment, horse bean meal with and without methionine, replaced soybean meal. The 15% horse bean diet gave slightly poorer results. Methionine improved performance from 20 to 50 kg, but not from 50 to 90 kg. In additional experiments with higher levels of horse bean meal, 30% reduced performance and 42.5% was unsatisfactory. When two varieties of horse bean meal were compared, there were no differences between them.

TABLE 6
HORSE BEANS (VICIA FABA) FOR SWINE

Raw vs. Basal	Heat treatment	Methionine Supplementation	Reference
Worse	-----	Improved	Aherne & McAleese (1964).
Equal	-----	-----	Navrátil (1965).
Worse	-----	Improved	Balboa (1966).
Worse	-----	Improved	Hansen & Clawsen (1969).

Table 6 summarizes the main results of feeding horse beans to swine.

Common Beans *(Phaseolus spp.)*.

The published information regarding the use of beans as swine feed is very scarce.

Cooking (steaming or boiling) is recommended as a way to improve the feeding value and palatability of beans. Also, their combination with a source of animal protein appear to be indicated. Beans can replace from 25 to 50% of the corn of growing-finishing pig rations and seem to give the best results when fed to well-grown swine (1, 20).

TABLE 7
RAW (R) AND TOASTED (T) LEGUME SEEDS FOR SWINE

Treatment		Total gain, kg	Feed/gain
Dried skim milk		5.63	1.70
Phaseolus aureus	R	3.46	2.63
	T	4.22	2.40
Vigna sinensis	R	2.53	3.89
	T	4.13	2.02
Phaseolus calcaratus	R	3.78	2.27
	T	4.50	2.10
Cajanus cajan	R	0.63	7.20
	T	0.63	6.13

Sevilla-Eusebio et al. (14).

The effect of heat on the feeding value of four legume seeds, mung bean *(Phaseolus aureus)*, cowpea *(Cajanus cajan)* and rice bean *(Phaseolus calcaratus)*, was studied (14). Beans were toasted at 80 C and fed to weanling pigs. A dried skim milk control diet was also included. All diets were isonitrogenous (16% protein) and were balanced with ground rice. Toasting improved performance in all seeds except for pigeon peas, which gave very low gains in both the raw and toasted form (Table 7). Digestibility of protein was improved with the heat treatment in all seeds except for pigeon peas. Performance and digestibility of all legumes were inferior to the basal diets.

Carob beans *(Ceratonia siliqua)*.

Carob beans were fed to growing pigs in a series of experiments (21). In all trials, the inclusion of the bean meal at the expense of corn, decreased performance as compared to the control diet.

Chickpeas *(Cicer arietinum)*.

Chickpeas were fed alone or combined with sorghum and compared with a standard sorghum-based diet (22). The chickpea-sorghum combination diet resulted in a significantly better performance of growing and finishing pigs.

Chickpeas were compared with corn-soy for growing swine. A 16% protein basal diet was used and the test diets included 22.2, 44.4, 66.6, and 88.8% chickpea substituting for equal amounts of corn-soybean meal; all diets were isonitrogenous. Eight week data showed similar performance at all levels of substitution (Table 8). Percent digestibiity of dry matter and energy followed a similar trend. The apparent digestibility of nitrogen was decreased with the increasing levels of chickpea. In a second experiment, the addition of graded levels of methionine (0, 0.1, 0.2, and 0.3%) to diets containing 88.8% chickpea did not significantly affect performance of growing pigs. Total gains were 32.2, 29.9, 33.3 and 28.3 kg, respectively; feed/gain ratios were 2.95, 2.93, 2.80 and 2.86, respectively (23).

TABLE 8
CHICKPEAS (CICER ARIETINUM) AND SOYBEAN MEAL IN SWINE DIETS

Chickpea, %	0	22.2	44.4	66.6	88.8
Soybean meal, %	20.0	15.0	10.0	5.0	0
Daily gain, kg	0.604	0.557	0.530	0.527	0.538
Feed/gain	3.20	3.33	3.48	3.40	3.57

Shimada & Brambila.
(23)

An experiment was conducted to evaluate the nutritive value of chickpea, either raw or autoclaved, with and without the addition of 0.2% DL-methionine. Weight gain and feed efficiency

of pigs fed raw or autoclaved chickpea were similar, which indicated that there was no significant effect of the heat treatment on growth. The addition of methionine improved gain and feed efficiency slightly, but the differences were not statistically significant (Table 9). A second experiment was conducted to determine the apparent digestibility coefficients for raw and autoclaved chickpea; heat-treatment consisted in autoclaving at 115 C for 60 min. or to use the same temperature for 30 min. The apparent digestibilities of dry matter (78.5, 69.4, 65.8%), energy (77.9, 68.3, 64.7%) and crude protein (70.0, 62.7, 66.6%) were higher for the raw chickpea diet than for the cooked counterparts. The digestible energy values obtained were 3.45, 2.86, and 2.85 Kcal./g, respectively. The carcass yield of pigs fed 88.8% chickpea was lower than that of animals on the corn-soy control (77.4 *vs.* 80.9%).

TABLE 9
COOKING AND METHIONINE ADDITION TO CHICKPEAS (CICER ARIETINUM)

Chickpea	Raw		Cooked	
Methionine	-	+	-	+
Daily gain, kg	0.557	0.620	0.602	0.620
Feed/gain	3.14	2.86	3.00	2.87

Shimada & Brambila. (24)

Also, the carcass quality obtained with the chickpea diet, as judged by the iodine number, was inferior. Dorsal backfat samples of 4 pigs fed chickpeas for 10 weeks had an iodine number of 66.8, whereas the value was of 61.7 for animals on the basal diet (24).

In another experiment, the effect of replacing 0, 50 and 100% of the fish meal of a basal sorghum (78.8%), cottonseed meal (12.5%) and fishmeal (6.2%) diet was studied (25). Performance of pigs was similar for all treatments and emphasized the high nutritional value of chickpeas and the possibility of all-vegetable protein diets for swine.

To determine the first limiting amino acid of chickpea for growing-finishing swine, diets with suboptimal levels of protein (but adequate in lysine) were fed. Performance of pigs fed chickpea

with 0.28% added DL-methionine was superior, which indicated that the sulphur amino acids are first limiting in chickpea. The availability of the lysine provided by chickpea protein was determined by a growth study and was found to be very high (26).

TABLE 10
CHICKPEAS (CICER ARIETINUM) FOR SWINE

Raw vs. Basal	Heat treatment	Methionine Supplementation	Reference
Equal	-----	-----	Flat (1965).
Equal	-----	no effect	Shimada & Brambila (1967).
-----	no effect	no effect	Shimada & Brambila (1968).
Equal	-----	-----	Shimada & Brambila (1968).
-----	-----	improved at low protein levels	Castrín & Bravo (1974).

Several combinations of chickpea and sorghum were fed to pigs from 35 to 60 kg. It was observed that pigs performed better when chickpea provided 75% and sorghum 25% of the dietary protein (26).

The experiments on the nutritive value of chickpea for swine are summarized in Table 10.

Peanuts *(Arachis hypogaea).*

Peanuts and soybeans are two legume seeds which store their energy in the form of oil, instead of carbohydrates as the rest of the seeds already covered. This unique characteristic allows the obtention of oil for human consumption and several by-products used mainly for animal feeding. Peanut meal has been used widely in swine feeding, and has been shown to be deficient in lysine for the growing pig (27, 28, 29).

The feeding of whole unextracted peanuts to swine has been practiced for a number of years in the Southern United States (1). There is however, very little modern scientific information about the nutritional value of such peanuts for swine.

When unextracted peanut were fed to growing pigs, cooking improved significantly consumption and thus performance over the uncooked controls. Performance of pigs fed shelled peanuts was better than that of animals fed whole peanuts (30).

The feeding of unextracted peanuts to swine increases the problem of "soft carcasses" due to an increase in the polyunsaturat-

ed fatty acids in the pig fats. Soft carcasses are undesirable to meat packers and processors, and to the consumers. Eighty-kilogram pigs were used to study the effect of feeding 10% whole peanuts for 4 weeks and then 20% whole peanuts for another 4 weeks, in the fatty acid composition of several tissues. It was found that C18:2 tends to increase in all organs and body tissues, although it was significant only in liver. C18:1 increased in liver while it decreased in other organs and tissues. In general, it was found that the polyunsaturated fatty acids content of all tissues changed very slightly despite the higher lipid content of the peanut diet (31).

Soybeans *(Glycine max)*.

This is probably the best known source of protein for swine. Most of the soybeans are fed in the form of soybean meal, a by-product of the soy-oil extracting industry. An extensive review of literature about soybean meal in swine feeding was recently presented (32).

In some cases, it seems that the use of whole unextracted soybeans in animal feeds could be economical.

The nutritional superiority of cooked soybeans over their raw counterparts was reported as early as 1935 (33).

When raw and heated soybeans were compared with a soybean meal-containing control diet, the use of raw soybeans decreased performance as compared to the controls. Heat-treating the soybeans significantly improved performance over the controls (Table 11).

TABLE 11
RAW AND COOKED SOYBEANS (GLYCINE MAX) FOR SWINE

Treatment	Daily gain, g	Feed/gain	Iodine number
Soybean meal	773[b]	3.56	73[a]
Soybean meal + oil	786[b]	2.97	88[b]
Cooked soybeans	841[c]	3.08	85[b]
Raw soybeans	611[a]	3.97	92[b]

abc Values in a column with the same superscript do not differ differ significantly ($P<.05$).
Jiménez et al. (34).

Similar results were obtained in a second experiment, in which it was also shown that the addition of methionine caused a significant effect on heated soybeans (659 *vs.* 745 g average daily gain) but not on the raw beans (313 *vs.* 336 g). In both experiments, the iodine numbers of pigs fed the experimental diets were significantly increased over the corn-soy control (34).

Graded levels of raw unextracted soybeans caused a linear decrease in weight gains in both growing and finishing pigs (Table 12) (35).

TABLE 12
RAW SOYBEANS (RSB) VS. SOYBEAN MEAL (SBM) FOR SWINE

RSB, %	0	5.40	10.80	16.45	22.15
SBM, %	17.85	13.50	9.15	4.60	0
Daily gain, kg.	0.73^{a}	0.73^{a}	0.64^{ab}	0.56^{b}	0.45^{c}
Feed/gain	3.11^{a}	3.19^{a}	3.25^{a}	3.64^{b}	4.32^{c}

abc Values in a row with the same superscript do not differ significantly ($P<.05$).
Young (35).

TABLE 13
RAW VS. HEATED SOYBEANS (GLYCINE MAX) FOR SWINE

Treatment	Daily gain, g	Feed/gain	Protein Digestibility %
Raw	190^{a}	4.38	44.4^{a}
Raw + fat	230^{a}	2.78	48.0^{ac}
Heated (15min.)	290^{a}	2.60	55.3^{bcd}
Heated (30min.)	500^{bc}	2.26	57.6^{bcd}
Heated (120min.)	550^{bcd}	1.58	68.0^{be}
Soybean meal	640^{bd}	1.99	61.3^{bde}

Values in a column with the same superscript do not differ significantly ($P<.05$).
Combs et al. (36).

Which soybeans were heat-treated, optimum response was obtained by prolonged heating at low pressure (110 - 0.68 kg/cm^2, 120 min.) (35) (Table 13). The results also showed that raw or heated soybeans suppressed the availability of certain amino acids.

Soybeans were cooked in water at 10 C for six hours and it was shown that the process improved the nutritional value of the diets to a level similar to a corn-soybean meal control (700 *vs.* 600 g average daily gain: 0.47 *vs.* 0.43 gain/feed; 82.3 *vs.* 86.6% protein digestibility: 41.7 *vs.* 44.1% protein retention). Pigs fed raw soybeans grew less (510 g; 0.35) and had a lower protein digestibility (73.0%) and retention (33.7%) (37).

The results on the nutritive value of soybean for swine are summarized on Table 14.

TABLE 14
SOYBEANS (GLYCINE MAX) FOR SWINE

Raw vs. Basal	Heat treatment	Methionine supplementation	Reference
Worse	Improved	-----	Jimenez et al. (1963).
Worse	Improved	Improved	Jimenez et al. (1963).
Worse	-----	-----	Young (1967).
Worse	Improved	-----	Combs et al. (1967).
Worse	Improved	-----	Young (1972).

SUMMARY

Cull legume seeds are used as sources of protein and energy in swine feeding. Peas (Pisum sativum and Pisum arvense) are good sources of supplementary protein for wheat and barley rations. Performance of pigs fed peas is equal or better than that of animals fed meat, fish, or soybean meals. When high levels of peas are used, performance decreases conversely. Cooking improves the nutritional value of peas. Cowpeas (Vigna sinensis) can be included as 50% of pig rations without detrimental effects on growth. Toasting or cooking of cowpeas significantly improves growth and digestibility, while germination depresses performance. The addition of methionine is beneficial only in the germinated cowpea diets. Horse beans (Vicia faba) can be included at low levels in swine rations without affecting performance; however, when the level of horse beans in the diet is rised above 20%, there is a significant growth depression. Horse bean protein is comparatively inferior to that of soybean, meat, and fish meals and similar to torula yeast's. The addition of methionine to horse bean rations significantly improves performance. Common beans (Phaseolus spp.) are a good source of protein for swine when they are cooked and combined with sources of animal protein. Common beans can replace from 25 to 60% of the corn of growing-finishing pig rations. Mung beans (Phaseolus aureus) and rice beans (Phaseolus calcaratus) are improved by

toasting, but performance of pigs fed them is still lower than that of swine fed dried skim milk. Carob beans (Ceratonia siliqua) included to swine rations at the expense of corn, decrease performance. Pigeon peas (Cajanus cajan), raw or toasted, severely depressed growth as compared to other beans. Chickpeas (Cicer arietinum) can replace sorghum, and soybean, cottonseed and fish meals without affecting pig performance. Cooking does not affect performance but decreases digestibility of chickpeas. The addition of methionine has little beneficial effect on growth but has a significant effect on feed conversion. Unextracted peanuts (Arachis hypogaea) and cooked unextracted soybeans (Glycine max) are excellent feeds for swine. The addition of methionine to cooked soybeans further improves their nutritional quality. Raw soybeans are not improved by sulphur amino acid supplementation. Feeding of unextracted peanuts and soybeans to swine results in soft pork.

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Pea fractions in milk replacers for calves

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INTRODUCCION

A major objective of much of the research that has been done on formulation of milk replacers for calves has been to develop substitutes for milk protein. Whole milk remains the most nutritious diet for newborn calves, following the colostrum feeding period, until they are several weeks of age. However, any acceptable reduction in the use of whole or skimmilk for calf feeding should increase the supply of milk for human consumption and possibly the economic returns to the dairy farmer.

The digestive enzyme capability of the calf has been studied extensively (10, 12) and found to be rather specifically adapted to a milk diet for the first few weeks of life. Preston (11) stated that there was little to be gained by adding alternate sources of protein to the diet of very young calves.

While attempts to replace milk protein with vegetable origin protein have generally failed Colvin and Ramsay (3, 4) obtained encouraging results with acid or alkali-treated soy flour. Gorrill and Thomas (6) and Gorrill and Nicholson (5) used soybean protein concentrate (71% crude protein) to supply 60% of the dietary protein and found this material to be well digested and capable of promoting satisfactory growth rates in young calves.

Field peas *(Pisum sativum)* are composed mostly of starch and protein, the latter averaging about 24% (N x 6.25). The protein is relatively low in methionine content and is readily separated as a protein concentrate (1) but when supplemented, it is of high quality according to protein quality studies with mice. This study pertains to the evaluation of pea protein concentrate and an enzyme-treated pea flour as ingredients in milk replacers.

MATERIALS AND METHODS

Experiment 1

Twelve Holstein-Friesian calves, six males and six females, were allotted at three days of age to three diets in a simple block design. The diets were a control milk-based formula, a diet containing 20% pea protein concentrate (PPC) plus cerelose (hydrolyzed corn starch, largely glucose) to compensate for the loss of lactose when dried skimmilk was replaced, and a diet containing pea flour hydrolyzed (HPF) with a glucoamylase to convert the starch to simpler sugars.

The PPC was prepared as described by Bell and Youngs (1). The HPF was prepared from peas which had been split in a Burr mill and added to water in a 1:4 ratio, by weight. The resultant slurry was passed through a Bauer plate refiner three times and then poured into a steam-jacketed reaction kettle, where a starch-thinning enzyme (HT-1000)[1] was added at 0.25% of the weight of the original flour. After one hour at 95°C, the mixture was cooled at 55°C and a glucoamylase (Diazyme)[1] was added at 0.5% of the original weight of pea flour. After four hours at 55°C, the material was dried on a double-drum drier, then in an air-draft oven at 50°C and finally ground in a Wiley mill.

The PPC and HPF were incorporated in milk replacers at levels of 20 and 48%, providing half of the dietary protein, at the expense of skimmilk (Table 1).

Dry milk replacer was reconstituted with warm water (1:8) immediately before feeding and Cr_2O_3 was added at 0.5% of the dry matter allowance. Calves were fed three times daily and were given 680 gm. of feed, dry basis, initially and allowances were adjusted upwards according to appetite.

Experiment 2

Milk replacers containing 7.7, 15.4 and 23.1% PPC in the dry matter were fed in three successive 7-day periods in a Latin square design to study the effects of calf's age on ability to digest pea protein and the effects of level of PPC on milk replacer utilization. Three replicates of three calves were used. The formulas (Table 2) were similar to the PPC diet in the previous experiment except that lactose was used instead of cerelose to provide the carbohydrate displaced by substituting PPC for dried skimmilk.

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TABLE 1
COMPOSITION OF MILK REPLACERS USED IN TRIAL 1

Ingredient or factor	Diet		
	Control	PPC + Cerelose	Hydrolyzed pea flour (HPF)
Premix† (kg)	25.0	25.0	25.0
Skimmilk, dried (kg)	37.0	9.0	10.0
Whey, dried (kg)	37.0	15.0	14.0
PPC (kg)	-	20.0	-
HPF (kg)	-	-	48.0
Cerelose (kg)	-	28.3	-
Methionine (g)	110	350	240
Aureo‡SP250 (g)	250	250	250
Choline (g)	-	134	115
Vitamin A (I.U.)	243,000	358,000	358,000
Vitamin D (I.U.)	44,000	88,000	88,000
Vitamin E (I.U.)	242,000	242,000	242,000
Vitamin B_{12} (mg)	2.9	2.9	2.9
ZnO (g)	2.2	4.4	4.4
$FeSO_4 \cdot 2H_2O$ (g)	1.1	1.1	-
$FeSO_4 \cdot 7H_2O$ (g)	-	-	32.6
$CuSO_4 \cdot 5H_2O$ (g)	3.3	0.44	0.44
$CaHPO_4 \cdot 2H_2O$ (kg)	-	1.56	1.34
$CaCO_3$ (g)	-	-	540
MnO (g)	-	3.85	3.30
Cobalt-iodized NaCl (g)	-	400	400
Protein, Nx6.25, (%)	25.3	24.5	26.8
Protein from peas (% of total)	-	50.2	52.0
Nitrogen-free extract (%)	52.6	55.6	54.0

†20% Brewers' dried yeast, 40% lard, 33% dried buttermilk and 7% dried whey; homogenized and spray dried.

‡A mixture of chlortetracycline, sulphamethazine and penicillin. Courtesy of Cyanamid of Canada Ltd.

Experiment 3

Since rennin is the major proteolytic gastric enzyme in the very young calf and since the gastric pH may be too high for optimal pepsin activity at this stage of life, an experiment was designed to study the proteolytic breakdown of PPC by rennin, pepsin and by abomasal contents obtained from a fistulated calf, each tested at various pH levels ranging from 3 to 6.

Crystalline pepsin was prepared according to Clark and Lubs (2) in 0.1% solution in a 0.05 M HCl-KCl buffer, pH 1.8. The final pH of the medium was 2.8.

TABLE 2
COMPOSITION OF MILK REPLACERS USED IN TRIAL 2

Ingredient or factor	Level of PPC		
	7.7%	15.4%	23.1%
Premix[1] (kg)	25.0	25.0	25.0
Whey, dried (kg)	31.0	23.2	23.2
Skimmilk, dried (kg)	25.0	25.0	17.3
Brewers' dried yeast (kg)	5.0	5.0	5.0
PPC (kg)	7.7	15.4	23.1
Lactose (kg)	5.5	5.5	5.5
Methionine (g)	100	100	100
Aureo SP250 (g)	250	250	250
Vitamin A (I.U.)	236,000	236,000	236,000
Vitamin D (I.U.)	44,000	44,000	44,000
Vitamin E (I.U.)	242,000	242,000	242,000
Vitamin B_{12} (mg)	1.2	1.2	1.2
Protein, Nx6.25, (%)	26.7	30.6	32.3
Protein from peas (% of total)	19	33	46
Nitrogen-free extract (%)	54.1	50.8	49.1

[1]40% lard, 60% skimmilk, by weight; homogenized and spray dried.

Rennin solution (1%) was made by dissolving rennin[1] in 0.2M sodium acetate buffer, pH 3.7 (13). The final pH of this mixture was 3.7.

Abomasal contents were obtained from a calf that had nursed for the first three days and was then fed a milk based milk replacer for 14 days. The calf was cannulated on day 5. The first daily collection of abomasal juices was taken on day 8, just prior to morning feedings. The material collected was filtered through Whatman No. 1 filter paper under vacuum before use in the *in vitro* system.

RESULTS AND DISCUSSION

Experiment 1

Calves fed the milk-based control diet digested about 90% of the dry matter in all test periods (Table 3). Calves fed either the PPC of HPF diets digested less dry matter ($P < .05$) throughout the test period, but the differences compared to the control diet may have been smaller as the calves grew older. These effects were not restricted to the protein component in the pea diets;

TABLE 3

COEFFICIENTS OF APPARENT DIGESTIBILITY FOR DRY MATTER, CRUDE PROTEIN, ENERGY, ETHER EXTRACT AND NITROGEN-FREE EXTRACT OF CONTROL, PPC + CERELOSE AND HPF DIETS (TRIAL 1)

	Diet	Calf age in days						Diet Means
		7-9	10-12	13-15	16-18	19-21	22-24	
		%	%	%	%	%	%	%
Dry matter	Control	92	89	87	90	90	93	90^{a}
	PPC + Cerelose	76^{b}	73^{b}	86^{a}	86^{a}	87^{a}	87^{a}	83^{b}
	HPF	69^{b}	70^{b}	81^{a}	82^{a}	83^{a}	82^{a}	78^{c}
Crude protein	Control	84	83	83	86	84	88	85^{a}
	PPC + Cerelose	50^{a}	49^{b}	74^{a}	77^{a}	79^{a}	79^{a}	68^{b}
	HPF	51^{b}	54^{b}	72^{a}	72^{a}	75^{a}	73^{a}	66^{b}
Energy	Control	91	90	89	90	90	92	90^{a}
	PPC + Cerelose	72^{b}	65^{b}	85^{a}	85^{a}	86^{a}	85^{a}	80^{b}
	HPF	66^{b}	69^{b}	81^{a}	81^{a}	83^{a}	82^{a}	77^{b}
Ether extract	Control	91	91	94	96	96	95	94^{a}
	PPC + Cerelose	65^{c}	48^{b}	83^{a}	82^{a}	82^{a}	84^{a}	74^{b}
	HPF	46^{b}	56^{b}	82^{a}	87^{a}	86^{a}	89^{a}	74^{b}
Nitrogen-free extract (N.-F.E.)	Control	97^{a}	95^{ab}	94^{b}	95^{ab}	95^{ab}	96^{ab}	95^{a}
	PPC + Cerelose	91^{b}	93^{ab}	94^{ab}	94^{ab}	94^{a}	94^{ab}	94^{b}
	HPF	84^{b}	84^{b}	87^{ab}	88^{ab}	89^{a}	88^{ab}	87^{c}

Means without superscripts, or with similar ones, are not significantly different, $P<0.05$.

Effect of calf age comparisons in drows; diet comporisons in last column. Significance of interaction means is not shown, that is, diet comparisons within age groups.

similar responses were obtained in regard to energy, ether extract and nitrogen-free extract although varying in magnitude.

The protein in PPC and HPF diets was only about 50% digestible for calves up to 14 days of age. Since these diets contained milk proteins, it is obvious that the pea protein itself was less than 50% digestible. If it is assumed that the milk-origin protein in the pea diets was as well digested as in the control diet, the pea protein digestibility may have been as low as 25%. However, the decreases observed in digestibility for the non-protein fractions, ordinarily well digested, indicates that the pea protein depressed digestibility in general, possibly associated with increased laxation, and hence it may not be correct to assume that the milk protein digestibility remained at 83-85% in the pea diets.

By similar calculations, it can be shown that pea protein was 65-70% digestible by calves between two and three weeks of age. Similar age-related increases in digestive ability in young calves were observed by Noller *et al* (8) also working with vegetable-origin proteins.

Carbohydrate digestibility in the HPF diet was lower than in the PPC diet, as indicated by dry matter and nitrogen-free extract (NFE) digestibility (Table 3). Cerelose (largely glucose) is well utilized by young calves and this sugar and lactose were the main carbohydrates in the PPC diet. There was only about 5% starch in this formula. Thus the PPC diet allowed 91% digestibility of carbohydrate, compared with 95% for the control diet containing lactose as the only carbohydrate and 84 to 89% for the HPF diet.

The HPF diet contained about 12% of carbohydrate more complex than glucose, comprising 4.25% of unhydrolyzed starch and 7.79% of unidentified complex carbohydrates. Thus the 84% digestibility of NFE confirms the well-established inability of the newborn calf to digest starch (12) but also indicates that the *in vitro* hydrolysis of starch rendered it readily digestible by the calf. It can also be concluded that the lower dry matter and energy digestibility coefficients derived from PPC and HPF diets are attributable mainly to the poor digestibility of the protein fraction and secondarily to the small amounts of starch and other complex carbohydrates in the two pea diets.

Experiment 2

The three diets containing 7.7, 15.4 and 23.1% PPC supplied approximately 11 and 46% of the dietary protein respectively. As a result of the increasing levels of PPC used, the digestibility of the dry matter decreased ($P < .05$) from 90 to 86% (Table 4). Similar trends were observed for all the major components of dry matter although the depression in ether extract digestibility was not significant ($P = .05$).

TABLE 4

COEFFICIENTS OF APPARENT DIGESTIBILITY FOR DRY MATTER, CRUDE PROTEIN, ENERGY, ETHER EXTRACT AND NITROGEN-FREE EXTRACT FOR DIETS CONTAINING THREE LEVELS OF PPC (TRIAL 2)

	PPC in diet	Calf age in days			PPC level Means
		7-10	14-17	21-24	
	%	%	%	%	%
Dry matter	7.7	84	93	92	90[a]
	15.4	84	92	93	90[a]
	23.1	78	89	91	86[b]
Mean		82[b]	91[a]	92[a]	--
Crude protein	7.7	67	87	87	80[ab]
	15.4	74	87	88	83[a]
	23.1	63	81	85	76[b]
Mean		68[b]	85[a]	87[a]	--
Energy	7.7	81	93	92	89[a]
	15.4	82	93	92	89[a]
	23.1	74	89	90	84[b]
Mean		79[b]	92[a]	92[a]	--
Ether extract	7.7	74	96	95	88
	15.4	69	95	94	86
	23.1	59	92	94	82
Mean		68[b]	95[a]	94[a]	--
Nitrogen-free extract	7.7	94	97	95	95[ab]
	15.4	96	97	96	96[a]
	23.1	92	95	96	94[b]
Mean		95	96	96	--

Means without superscripts, or with similar ones, are not significantly different, $P<0.05$.

Calves showed improved digestive ability with increasing age, in terms of dry matter, crude protein, energy and ether extract, but not with NFE which was high initially and remained high. The differences between age groups of calves in response to levels of PPC were not significant ($P = .05$). However, with digestibility of crude protein and ether extract being 68% in this experiment for the youngest calves (Table 4) compared to 91 and 84%, respectively, for these components of the milk-based

control diet, the low digestibility of PPC for calves under 7 to 10 days of age was confirmed. The residual starch in the PPC did not appear to have been especially deleterious, although it is possible that the starch along with the indigestible PPC protein may have interfered with fat digestion, perhaps by encouraging bacterial action in the intestinal tract and shortening passage time of the digesta.

Experiment 3

The pH proteolytic activity curve (Figure 1) clearly indicated that the susceptability of pea proteins to proteolysis by rennin, pepsin and abomasal contents was remarkably affected by pH; the degree of hydrolysis increased with decreasing pH. It was also evident that the decline in proteolysis by abomasal contents accelerated with increases in pH above 4.5. The degree of breakdown was found to decrease by 38 and 87% at pH 5.3 and 5.7, respectively, while at a lower pH of 4.5 the degree of proteolysis

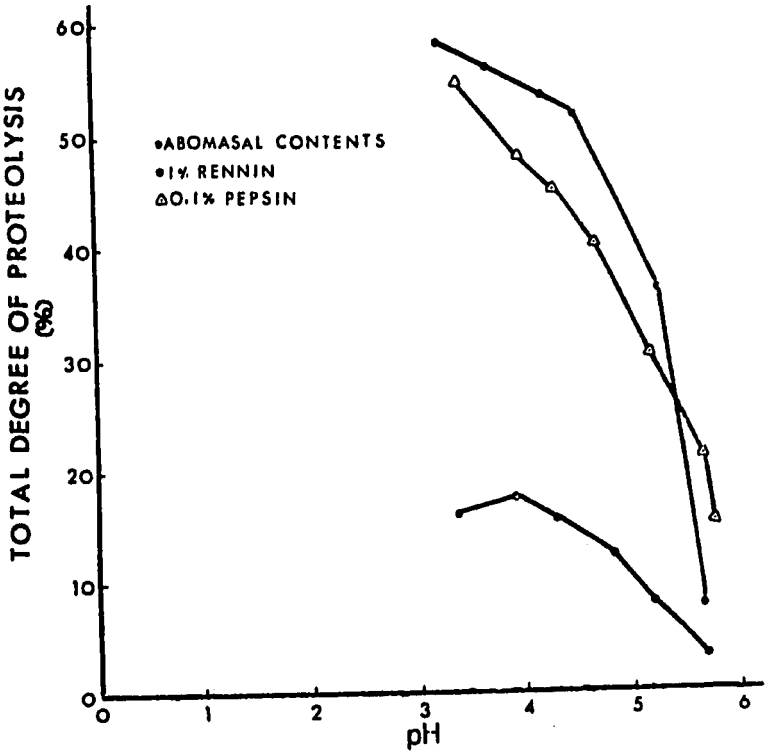

Figure 1. Effect of pH on the degree of proteolysis of PPC in vitro, using three enzyme sources.

was decreased only by 11%, compared to that at pH 3.3. This observation is important in view of the finding that the post-feeding pH of abomasal contents observed after whole milk feeding differs from that after milk replacer feeding. This may assist in explaining why pea protein was not well digested by newborn calves.

CONCLUSIONS

These studies indicte that PPC and HPF, as prepared for these experiments, are not well enough digested by calves under 14 days of age to be recommended as ingredients in milk replacers for such calves. However, the increased digestive powers of calves at 2 to 3 weeks of age resulted in 70 and 80% digestibility of protein and starch, respectively, and hence such pea products may be useful in formulas for older calves.

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SUMMARY

Field Peas (Pisum sativum) were fractionated into a protein concentrate (PPC: 60% protein) and a starch-rich fraction. The PPC was included in a milk replacer as 20% of the formula, providing half of the dietary protein, substituting for milk protein. Pea flour made from dehulled peas was hydrolyzed in vitro using a commercially available glucoamylase to convert the pea starch to simpler sugars. This hydrolyzed pea flour (HPF) was also used in a milk replacer, likewise replacing half of the milk protein. These diets, and a milk-based control diet, were fed to calves 3 to 21 days of age to determine digestibility of energy and protein. The protein in HPF and PPC was about 25% digestible by calves under 14 days old and 65 to 70% digestible thereafter. Calves under 10 days old digested 42 to 53% of the pea carbohydrate, older calves digested up to 84%. In a second experiment PPC was used a 7.7., 15.4 and 23.1% of the dietary dry matter. Protein digestibility declined with increasing level of PPC, especially with calves under 10 days old, but digestibility of all protein and energy fractions exceeded 80% with calves over 14 days old. In order to study the causes of the poor digestibility of PPC by young calves, PPC was subjected to in vitro hydrolysis by each of rennin, pepsin and abomasal contents obtained from a newborn calf equipped with a gastric fistula. Rennin

was less effective than pepsin or abomasal contents. The pH of abomasal contents was found to be too high for optimum pepsin activity. Pea protein and pea starch are too indigestible for satisfactory use in milk replacers for calves under 10 to 14 days of age, but may be useful for older calves.

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Nutritional value of different varieties of beans (Phaseolus vulgaris) and cowpeas (Vigna sinensis) for chicks and factors affecting nutritional value

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INTRODUCTION

It appears to be commonly accepted that properly cooked common beans *(Phaseolus vulgaris)* have an unusually high nutritional value for most animals after a deficiency of the sulfur amino acids, primarily methionine, is corrected. The evidence, at least for the chick, would indicate that the nutritional value of other grain legumes such as cowpeas, soybeans and common peas, when properly processed and supplemented with methionine, is much higher than the nutritional value of common beans. Riley (1) found that cooked beans failed to support good growth of young chicks after cooking, even after the methionine deficiency was corrected, and also that the metabolizable energy value of cooked common beans was considerably lower than would be expected based on the proximate analysis of beans. Goatcher and McGinnis (2) found that the nutritional value of cooked beans for the young chick was markedly improved when any one of a number of antibiotic supplements was added to the diet. This finding, plus the observation that a high level of cooked beans in the chick diet causes a severe diarrhea, suggests that beans modify the intestinal microflora in the young chick in an undesirable or harmful way which may in turn cause severe growth depression. In addition to causing the diarrhea, a high dietary level of cooked beans causes the chick feces to adhere tenaciously to the wire screen floor and to the anal area of the chick.

In the course of studies for an advanced degree during the past two years, Capella (3) has investigated the effect of treating cooked beans in different ways and fractionating beans by different procedures on their nutritional value for chicks. Data obtained in a number of these expriments will be presented.

GENERAL PROCEDURE

In most of the trials to be cited, the data were collected during a two-week growing period from hatching to two weeks of age. Commercial heavy breed broiler type chicks, usually of the male sex, were used in these studies. Unless otherwise noted, three of four replicate groups of 10 chicks were used per treatment. The experimental diets, except as indicated in the experimental outline, were intended to be nutritionally adequate and for this reason in most trials supplemental methionine was used and diets that, at least on a theoretical basis, were adequate in other amino acids, vitamins, minerals and energy were fed. The results obtained in most of these trials, however, would indicate that this most certainly was not the case when the growth data obtained were compared with control type diets.

The cooking process for the beans consisted of autoclaving either ground or unground beans in trays approximately 2 cm deep for a period of 30 minutes at 121°C. This same process was followed when other test materials such as lima beans, cowpeas, or fractions of common beans were subjected to cooking.

In many of the trials the experimental diets were fed with and without a supplement of procaine-penicillin. When penicillin was used as an experimental variable, it was added to diets at a level of 50 ppm in the form of the procaine salt.

Influence on Growth of Bean Level in Diets

Because of the likelihood that variation in experimental results may be related to factors supplied by beans in the diet, aside from protein, a trial was conducted to determine the effect of two different levels of dietary protein and two different proportions of protein from beans on the results obtained. The diets used in these studies were in all cases supplemented with methionine. Penicillin was fed as a variable. The results obtained in this trial are shown in Table 1.

It can be seen from these results with the high level of protein, and 2/3 of this level coming from bean, that chick growth was greatly depressed. In every comparison at both levels of protein and both proportions, the soybean meal control diet gave markedly superior growth to the diets containing beans. Addition of penicillin to all diets gave increased chick growth.

Comparative Nutritional Value of Beans, Lima Beans and Cowpeas

The results obtained in the feeding trial where different varieties

TABLE 1
EFFECT OF DIETARY PROTEIN LEVEL AND PROPORTION DERIVED FROM BEANS ON CHICK GROWTH AND RESPONSE TO PENICILLIN

DIETARY PROTEIN %	PROPORTION FROM BEANS	PENICILLIN SUPPLEMENT	AV. CHICK WEIGHT 2 wks.
23	1/2	-	158
		+	173
23	2/3	-	93
		+	122
23	Soybean control	-	193
		+	249
18	1/2	-	153
		+	169
18	2/3	-	130
		+	161
18	Soybean control	-	179
			195

of common beans were compared and where two different types of lima beans and one variety of cowpeas were used are shown in Table 2.

All of the test materials used in this trial were subjected to an autoclaving cooking treatment and methionine was used as an experimental variable. Growth of chicks fed all of the different varieties of common beans was increased with the supplemental methionine but in the case of some of the varieties the increase was only slight. The combination of methionine and penicillin further improved chick growth with all varieties and tended to

TABLE 2
VARIETAL RESPONSE IN BEANS, LIMA BEANS AND COWPEAS AND RESPONSE TO METHIONINE AND PENICILLIN

VARIETY	AV. CHICK WT. AT 2 WEEKS		
	NONE	.3% METH.	0.3 METH + PENICILLIN
SOYBEAN MEAL	182	201	232
RED MEXICAN	150	167	180
PINK	161	161	184
LIGHT RED KIDNEY	160	175	185
DARK RED KIDNEY	152	162	184
PINTO	162	179	190
SMALL WHITE	153	158	180
FLAT WHITE	170	178	194
NAVY	167	175	192
GREAT NORTHERN	162	167	194
BLACK TURTLE	159	168	183
BABY LIMA	170	183	209
LARGE LIMA	169	183	211
BLACKEYE PEAS	170	188	210

All diets contained 50% of test material and approximately 23% protein.

reduce the differences among varieties. The two varieties of lima beans and the sample of cowpeas used gave similar results to common beans without a supplement of methionine, but when methionine or the combination of methionine and penicillin was added, these legumes supported growth which was superior to that given by the common beans. This was especially striking with the combination supplement. It should also be noted that soybean meal gave much better chick growth than any of the common beans.

The data obtained in another trial, where varieties were compared, are presented in Table 3. In this trial all of the test materials were subjected to a cooking treatment except the blackeye peas in one treatment. The results show a marked response to autoclaving of the blackeye peas, and again all of the materials responded

markedly to supplemental methionine and penicillin and to the combination. Again, lima beans and autoclaved blackeye peas supported better chick growth than the two varieties of common beans that were fed.

TABLE 3
COMPARATIVE NUTRITIONAL VALUE OF SOME DIFFERENT BEANS AND EFFECT OF AUTOCLAVING ON VALUE OF COWPEAS

TEST MATERIAL	AUTO-CLAVED	AV. CHICK WTS. - gms (2 wks)			
		NONE	METH.	PENICILLIN	BOTH
SOYBEAN MEAL	---	128	168	143	177
RED MEXICAN	YES	88	121	101	138
FLAT WHITE	YES	90	141	107	146
BABY LIMA	YES	97	142	112	164
BLACKEYE PEAS	YES	116	162	133	162
" "	NO	97	129	106	136

18% protein with 2/3 from test material.

A striking difference in the effect of common beans and lima beans and cowpeas on diarrhea and the type of feces was observed in these two trials. In contrast to the common beans, the lima beans and cowpeas did not cause diarrhea and the feces did not adhere to the wire screen floors.

Nutritional Value of Different Selections of Cowpeas

Five different samples of cowpeas obtained from Nigeria (IITA) and one sample of blackeye peas grown in California were fed in a chick trial, with and without a methionine supplement. The number of replicate groups per experimental treatment differed because of limited quantities of test materials. The results obtained in this trial are shown in Table 4. All selections were greatly improved with a methionine supplement as was soybean meal. The interesting point here is the small difference obtained between some of the varieties of cowpeas and soybean meal, with this difference being much smaller than that usually obtained in a comparison of soybean meal and common beans. This is particularly true when 2/3 of the dietary protein is supplied by the test material, as was done in this trial.

TABLE 4
COMPARATIVE NUTRITIONAL VALUE OF DIFFERENT SELECTIONS OR VARIETIES OF COWPEAS

TEXT MATERIAL		CHICK WT. AT 2 WKS.--Gms. NONE	+ METHIONINE
SOYBEAN MEAL		124	183
BLACKEYE PEAS		115	164
Ivu 32	AFRICA	109	173
" 37	AFRICA	112	173
" 57	AFRICA	118	173
" 176	AFRICA	125*	159*
COWPEAS	AFRICA	113	168

Diets contained 18% protein-2/3 from test material.
* Two replicates.

Effect of Processing and Fractionation of Beans on Their Nutritional Value

Because of the greatly reduced chick growth that was obtained in a number of experiments where cooked beans were fed with supplemental methionine in comparison with growth of chicks fed soybean meal, it was surmised that some factor is present in beans other than limiting or deficient amino acids which causes the poor growth. This suggestion was further strengthened by the observation that antibiotic supplements give such a large growth response when added to diets with beans, and because of the diarrhea problem encountered when high levels of beans are fed. A number of different experiments were conducted to determine the effect of processing and fractionating beans in different ways on their value for chicks.

The results of a trial in which the autoclaved or cooked beans were further cooked after wetting with an alkaline solution are shown in Table 5. In this trial the ground, autoclaved beans were mixed with approximately an equal weight of dilute sodium hydroxide solution and the pH was adjusted then by adding concentrated NaOH until a value of 8.8 was obtained. The beans were then again autoclaved and spread on paper and air-dried before being reground and mixed in the diets. The results of this trial show

that the alkaline treatment of the beans gave a further and significant improvement in chick growth. The nutritional value was, however, still lower than the value of soybean meal.

TABLE 5
EFFECT OF ALKALINE AUTOCLAVING BEANS ON NUTRITIONAL VALUE FOR CHICKS

TREATMENT OF BEANS*	AV. CHICK WT. (gm) 2 WKS
AUTOCLAVED	192 a
ALKALINE AUTOCLAVED**	212 b
SOYBEAN CONTROL	239 c

* Diet contained 50% red beans.
** pH adjusted to 8.8 before cooking.

An additional experiment was conducted to further investigate the effect of alkaline cooking of beans on their nutritional value and on the response to a dietary penicillin supplement. The results of this trial are presented in Table 6. The alkaline cooking of the beans again gave an increase in chick growth and in all treatments a further response was obtained by addition of a penicillin supplement to the diet. These results indicate that the factor which is destroyed or inactivated by the alkaline cooking treatment is unrelated to the factor which causes a growth response to a penicillin supplement.

Because of the reports (4) which indicate that resorcinols or phenolic compounds in the seed coat of beans and other grains may have growth depressing effects, an experiment was conducted with beans subjected to acetone extraction to remove phenolic substances present. In this trial the cooked and ground beans were subjected to continuous acetone extraction for approximately 24 hours before being dried and mixed in the feed. The results obtained in this trial are shown in Table 7. It can be seen from the growth data that acetone extraction had no influence on the nutritional value of beans and, further, that addition of the acetone extract of beans to the control diet had no influence on growth of

chicks fed this diet. Similar results were obtained in the series, with and without a penicillin supplement.

Following the experiment in which acetone extraction failed to improve the nutritional value of cooked beans, it was decided to determine whether water extraction of ground autoclaved beans or soaking whole beans in water before autoclaving would improve their nutritional value. In this trial the water soluble extract

TABLE 6
EFFECT OF pH AND AUTOCLAVING ON NUTRITIONAL VALUE OF BEANS AND THE GROWTH RESPONSE TO PENICILLIN

BEAN TREATMENT	PENICILLIN SUPP.	AV. CHICK WT. 2 WKS
AUTOCLAVED	-	154
	+	180
ALKALINE AUTOCLAVED	-	188
	+	204
SOYBEAN CONTROL	-	214
	+	224

TABLE 7
GROWTH OF CHICKS FED DIETS CONTAINING ACETONE EXTRACTED BEANS
(Weight at 2 wks of age)

Treatment	Supplement	
	None	Proc. Pen.
Control	191	205
Beans	147	176
Acetone extracted beans	147	173
Control + acetone extract of beans	182	202

obtained by stirring the ground beans in water and centrituging, and the water soluble material removed by soaking beans in an excess of water followed by decanting the aqueous phase, was discarded. The results obtained in this trial are shown in Table 8. No significant improvement in the nutritional value of cooked beans was obtained either by water extraction of the ground beans or by soaking the whole beans in an excess of water before cooking.

TABLE 8
EFFECT OF WATER EXTRACTING AND WATER SOAKING BEANS ON CHICK GROWTH

BEAN TREATMENT	AV. CHICK WT.-gms, 2 weeks
WHOLE BEANS AUTOCLAVED	199
GROUND BEANS WATER EXTRACTED (10 BEANS: 1 WATER - 2X)	217
WHOLE BEANS SOAKED IN EXCESS WATER 12 hrs-3X	202

All diets contained penicillin.

Because of the possibility that a growth depressing factor may be present in the seed coats and is neither soluble in acetone or water, an experiment was conducted to determine the effect of removing the seed coat of red beans on their nutritional value for chicks. In this trial, the uncooked whole beans were soaked in water to soften the seed coats and the soaked beans were then stirred in a Hobart food mixer with a whip. Most of the seed coats separated from the seed in this process and were decanted off with the aqueous phase. The remaining seed coats were removed by hand. The beans were then either subjected to autoclaving before drying or the pH was adjusted as indicated in previous trials and autoclaved before drying and grinding. The results obtained in this trial are shown in Table 9. It can be seen from the chick weight at 2 weeks of age that removing the seed coats had no effect on nutritional value of the beans, and it can be seen further that the alkaline autoclaving treatment gave essentially the same improvement in value when whole ground beans were processed or beans from

hich the seed coats had been removed were treated. Again the ybean meal control supported much better chick growth than any the treatments containing cooked beans. This difference, however, as smaller when beans were subjected to an alkaline cooking eatment.

TABLE 9
INFLUENCE OF REMOVING SEED COAT AND ALKALINE AUTOCLAVING BEANS ON CHICK GROWTH

TREATMENT OF BEANS	2-WEEK AV. CHICK WT., gm.
AUTOCLAVED	202
SEED COAT REMOVED BY WATER SOAKING	204
ALKALINE AUTOCLAVED	237
SEED COAT REMOVED AND ALKALINE AUTOCLAVED	231
SOYBEAN CONTROL	263

All diets contained penicillin.

fluence of Animal Age on Nutritional Value of Beans

There have been a number of reports in the scientific literature owing that the response to cooking raw soybeans in nutritional lue is greatly reduced as animals grow older, and that essentially response is obtained in adult chickens after a deficiency of ethionine in the raw soybean meal is corrected. Results obtained rat experiments showed that a mixture of amino acids combined th an antibiotic supplement and added to a diet containing raw ybean meal gave growth that was equal to that obtained with diet containing cooked soybean meal and supplemented with ethionine.

Two experiments were conducted with mature laying hens to dy the effect of cooking raw beans and of adding penicillin to ts containing beans on egg production and feed consumption. In se trials, a level of approximately 50% raw or cooked beans

as used in an otherwise practical type laying ration. The results f the first trial are shown in Table 10. In this trial, raw beans were d with and without penicillin and cooked beans were fed with enicillin. Egg production results showed a marked drop, even uring the first week on experimental diets and all hens fed raw eans were essentially out of production by the end of the first vo weeks. The treatment containing cooked beans and penicillin ave results that were comparable in egg production with the control et, but feed consumption was increased.

TABLE 10
EGG PRODUCTION AND FEED CONSUMPTION OF HENS FED RAW AND AUTOCLAVED BEANS

Treatment	Egg Prod. %	Feed Consump. gm/day/hen
Control	67	107
Raw red cull beans	15	86
Raw red cull beans + penicillin[1]	16	95
Autoclaved red cull beans penicillin	70	126

[1] Procaine penicillin supplement of 50 ppm

Results of the previous trial with laying hens did not make possible to conclude whether an antibiotic supplement is required r maximum utilization of cooked beans by laying hens, as is the se for the young growing chick. A second laying hen trial was, erefore, conducted and the results of this trial are shown in able 11. Egg production was the same for all treatments, showing at cooked beans have a higher relative nutritional value to ybean meal for adult birds than they do for young chicks and so that penicillin was not essential for maximum egg production, en when the diet contained beans. Again, hens fed the diet ntaining cooked beans consumed more feed than those fed the ntrol diet.

TABLE 11

EGG PRODUCTION, EGG WEIGHT AND FEED CONSUMPTION OF HENS FED A DIET CONTAINING A HIGH LEVEL OF AUTOCLAVED BEANS

Treatment	Egg Prod. %	Egg Weight gm	Feed Consumed gm/hen/day
Control	89	55	110
Autoclaved red cull beans	89	55	128
Autoclaved red cull beans + penicillin	89	56	117

SUMMARY

The results concerning the nutritional value of different varieties of common beans and some other grain legumes and of processing treatments of the beans may be briefly summarized as follows:

1. Some significant differences in nutritional value among varieties of Phaseolus vulgaris were obtained in feeding trials with chicks.

2. Nutritional value of essentially all varieties of beans was improved by addition of a methionine supplement, a penicillin supplement, and a combination of these. The differences among the varieties tested were greatly reduced after supplementation with a combination of methionine and penicillin.

3. The nutritional value of two different types of lima beans and of a number of selections or varieties of cowpeas was considerably higher for chicks than was the value of common beans in the same trial.

4. Diets containing high levels of cooked, common beans caused a marked diarrhea in chicks and the elimination of feces that adhere strongly to the wire-screen floors.

5. Extraction of beans with either water or acetone failed to improve their nutritional value for chicks.

6. An alkaline autoclaving of beans, either before of after removal of the seed coat, gave a marked improvement in nutritional value, and this nutritional value was further improved by a penicillin supplement.

7. Mature laying hens appear to have a greater ability to utilize cooked beans than do young growing chicks, and the results with laying hens further indicate that addition of methionine and penicillin to a laying diet containing raw beans does not improve the value of raw beans. Cooking is required.

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Effect of storage on cooking qualities, processing, and nutritive value of beans

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Dry legume seeds, like the cereals and nuts, are regarded as nonperishable foodstuffs. This is in contrast to the flesh foods, milk, most fresh fruits and vegetables, and other classes of foods which are generally highly perishable unless processed in some way to preserve them. The relative stability of pulses and cereals in storage is a primary reason that these crops are the basic foodstuffs throughout much of the world. However, it must be remembered that their storage stability is only relative, not absolute. The purpose of this paper is to review what we know now —and what we don't know— about the effects of storage under various conditions on those characteristics of beans and other legume seeds important to their use as foods. In addition, data are presented for certain nutritional properties of beans after prolonged storage at low and moderate moisture contents.

Hardshell

When beans are prepared for eating, they are (1) rehydrated and (2) cooked. These are two separate phenomena although in some methods of preparation they are carried out simultaneously. Rehydration is the physical imbibition of water, whereas cooking involves such physical and chemical changes as the destruction of toxic factors, gelation of starch, and denaturation of proteins.

When a sample of beans is soaked in water at room temperature, it often happens that some of the units do not imbibe water, presumably because the seedcoats are impermeable. In the food technology literature this is referred to as "hardshell"; seedsmen

and others concerned with the germination of beans usually call it "seed hardness" but they appear to be the same phenomenon. Morris *et al.* (1) tested 12 varieties and strains of common beans by soaking samples for 24 hours at room temperature. The percentage of hardshells ranged from 1% to 45%. Gloyer (2) showed that the lower the humidity of the storage atmosphere, the higher the percentage hardshell beans and that the development of hardshell is reversible. He also made the curious observation that hardshell beans recover their ability to rehydrate well more quickly if exposed to high humidity air than if soaked in liquid water at room temperature.

As a practical matter the hardshell problem can be very largely or entirely overcome if beans are boiled or exposed to steam for a few minutes at the start of the soaking period. Furthermore, Bourne (3) showed that hardshell beans tend to be smaller than normal ones even in the dry state and this size differential is markedly increased after soaking. He suggests that the processor can eliminate hardshells by screening the soaked beans and discarding the very small sizes.

Cooking Time

At best dry beans require rather long cooking and it is now well established that after prolonged storage under unfavorable conditions, the time required to cook them tender may be greatly extended. This is a problem even to those who live in modern cities; it is obviously more serious where fuel is scarce and must be gathered by hand.

The loss of cookability, i.e., the increase in required cooking time, is a function of time, temperature, and moisture content during storage. Since a detailed review of research on the phenomenon was recently published (4), the principal findings will be discussed only briefly here.

Morris and Wood (5) adjusted samples of seven varieties of beans (*Phaseolus vulgaris* and *P. lunatus)* to different moisture levels and stored them at 25°C. Periodically they withdrew subsamples, cooked them for times established as adequate at the beginning of the experiment, and submitted them to a taste panel. In as little as 6 months beans stored at a moisture content of 13% or higher had a significantly firmer texture than those stored at low moisture; in other words, the higher moisture samples needed longer cooking to become tender.

Mattea (6) measured the required cooking times of several species and varieties of dry beans grown at various locations in the United States. These had been stored at room temperatue in a laboratory for 18 months. Most of the cooking time data were not very startling but pinto beans and Michelite beans grown in Michigan took almost 4 hours to cook tender, whereas the same varieties grown in Idaho cooked in 82 and 59 minutes respectively. Examination of the chemical data revealed that all lots of beans in the study had moisture contents below 10% except the Michigan samples, which contained about 13%.

These clues led us to undertake an extensive study of the effects of time, temperature and moisture content on required cooking times for pinto and Sanilac beans *(P. vulgaris)* and large Limas *(P. lunatus)* harvested in 1961 and in 1963 (7, 8). After sublots of the beans were adjusted to moisture levels in the range 6.5% to 16.7% they were stored at temperatures of 21 and 32°C (1961) or 4.5, 13, and 21°C (1963). Periodically samples were withdrawn, blanched and presoaked. Then their median cooking times were measured by an objective procedure.

The results of this study showed that beans deteriorate in cookability to a very serious degree under storage condition which might sometimes be encountered. For example, the cooking time of pinto beans at 14.4% moisture held for 7 months at 32°C showed a 14-fold increase. At the quite moderate temperature of 21° Limas of 15.5% moisture required 5 times as long to cook after 12 months as they had initially. Under the same conditions, Lima beans of 13.1% moisture doubled in required cooking time. Cooking time tripled for Sanilac beans containing 16.0% moisture when held for a year at 21°.

All three types of beans were fairly stable for a year if stored at 21° or below and at a moisture content of 10-11% or less. Differences in response for a given type of beans from the two crop years were minimal. Storage which greatly impaired the cookability of beans did not impair their rate of water imbibition at 45°C.

The moisture levels used in the above study were by no means unusually high for beans grown where summers are somewhat humid. The two lots of Sanilac beans were grown in Michigan and contained 17% moisture on arrival in California. Furthermore, the United States Standards for Beans (9) permit beans to contain up to 18% moisture without special labeling.

Rockland and Metzler (10) found that their quick-cooking Lima beans, made by infiltrating dry beans with a solution of various salts and redrying them, also took longer to cook after they were stored at an elevated temperature. Higher moisture content accelerated the change in cookability.

It must be concluded that in some areas of the world common storage of beans can lead to serious impairment in their cooking quality. If capital is available, this deterioration could be overcome by artificially drying beans to a moderate moisture level or by using mechanical refrigeration in storage facilities during the warm months.

Gloyer (11) quotes Theophrastus, who wrote in the third century B. C. "In the district of Philippi, if the beans while winnowed are caught by the prevailing wind of the country, they become uncookable, having previously been cookable". One may wonder whether the change referred to was due to the development of hardshell character or a true increase in cooking time due to high moisture storage. Two facts point clearly to the answer. At harvest time in southern Macedonia dry winds from the north prevail and this would tend to lower the moisture content of the beans. Secondly, the implication is that the change took place in a matter of hours or days, not months or years. Thus, the problem was undoubtedly one of hardshell. Apparently the ancient Greeks did not know the simple trick of blanching beans briefly before soaking them.

Nutrients - Proteins

The legume seeds commonly eaten by man are, of course, relatively high in protein content. In addition, they are an excellent source of thiamine and contribute appreciable quantities of the water-soluble vitamins, riboflavin, niacin, pyridoxine, and folacin, and of the minerals phosphorus, iron, calcium, and magnesium. (It should be pointed out, however, that much of the phosphorus is in the form of phytic acid, which can bind the other minerals in insoluble form and make them unavailable to the body).

The literature on the effects of storage on the nutritional values of legume seeds is quite limited and often leaves important questions unanswered.

Mitchell and Beadles (12) stored soybeans at 25.5°C for up to 34 months. Half the samples were ground, defatted, autoclaved, and dehydrated to 6.2% moisture before storage; the other half were stored as whole raw beans (6.6% moisture) and ground,

defatted, and autoclaved after storage. The latter samples, stored whole, showed a drop in their nitrogen digestibility by rats from 85.3% to 75.0% and the biological value of the protein fell from 69.1% to 56.0%. Combined, these represent a 28% loss in the nutritive value of the protein.

The beans stored in the ground, preheated state showed a much smaller loss of protein nutritive value, 7.5%.

Supplementation of the two materials with methionine increased the biological value in the beans stored raw by 6% and in the preheated sample by 20%. These facts led the authors to the conclusions "that the damage inflicted by storage on the metabolic utilization of soybean proteins relates primarily to the cystine or methionine (or both) component... and that the greater storage damage in the unheated beans involves also other amino acids than cystine or methionine".

It should be pointed out that storage of both lots was at a very low moisture content. Even if we assume that no moisture was associated with the 21% of lipids, the moisture content of the non-lipid fraction would only amount to 8.3% in the raw beans.

Changes in amino acid composition in dry peas were followed by Schuphan (13) during storage in a ventilated warehouse. Temperatures and moisture contents (or relative humidities) are not mentioned. During a 12-month period the sum of 10 essential amino acids fell 20%; lysine content fell 21%; and methionine decreased by 32%.

The loss of available lysine during the heating or storage of foods is attributed to the blocking of its epsilon-amino group by reaction with reducing sugars, with carbonyl compounds formed during the oxidation of fats, or with the free carboxyl groups of glutamic and aspartic acids. How rapid this can be is illustrated by an experiment of Ben-Gera and Zimmermann (14). They prepared a crude protein concentrate from raw defatted soybean flakes, air-dried it to 9.1% moisture content, and held it at 30°C. In 12 weeks the available lysine declined by 18%. Note that again the product was held at a very moderate moisture level, far below that at which beans are sometimes stored.

In more recent studies Yannai and Zimmermann (15) stored soybeans *(Glycine max)* at 20°, and 40°C in cabinets maintained at 60% relative humidity. Controls were held in vacuum at —18°C. Following storage the beans were autoclaved and fed to rats

as part of a diet adjusted so that lysine, rather than methionine, was the first limiting amino acid. After two years at the higher temperature, the protein efficiency ratio (PER) had fallen to 77% that of the control, which indicates a loss of available lysine. In a rather similar experiment (16) the authors stored autoclaved soybeans and peanut *(Arachis hypogaea)* meals for two years at both 40% and 60% relative humidities. For the soybean meal the decline in PER was not significant. However, for the peanut meal stored at 40° and the higher humidity the PER was 80% that of the control.

Nutrients - Vitamins

Dawson *et al.* (17) stored Michigan pea beans in glass jars at 4.5°C and in burlap bags at 24°C and a relative humidity varying between 67% and 95%. At the end of one year the 4.5° sample contained 11.0% moisture and 0.94 mg/100 g thiamine; the 24° sample had fallen to 8.2% moisture and 0.76 mg/100 g thiamine. Thus the beans had lost nearly 20% of their thiamine in a year at room temperature in spite of their relatively low moisture content.

Chitre *et al.* (18) stored pulses in amber-colored sealed bottles and in burlap sacks for six months. They found no significant loss of thiamine or of niacin. Riboflavin, on the other hand, increased in the sample stored in bags from an initial value of 132.5 ± 9.13 microg. per 100 g to a final value of 186.6 ± 20.0. The material stored in amber bottles showed no change. They also found increases in the riboflavin content of some samples of rice and other cereals during storage. Unfortunately, the authors failed to mention what kinds of pulses they studied, the temperature of storage, or moisture content of their samples.

Water is well known to be a catalyst for many chemical and enzymatic reactions. Furthermore, it has been established that in the storage of beans a number of changes proceed more rapidly at higher moisture levels. These include: decline in catalase activity, decline in phosphatase activity, increase in lipid acid values, increased respiration, impairment of flavor, impairment of texture for constant cooking time (5); increase in required cooking time, darkening of color (8); loss of germination capacity (19). All these changes occur at moisture contents well below the 18% permitted by the U. S. Standards for Beans (9), which, incidentally, is approximately the minimum for mold growth (20).

In view of these facts it seems quite possible that moisture content might have a significant effect on changes in protein quality and retention of vitamins during the storage of pulses. A study of this question has been initiated with 1973 crop beans but there are no results to report at this time.

Three small samples of 1963 crop pinto beans which had been used in our earlier study of cookability (8) were available. They had originally been adjusted to moisture contents of 8.2%, 12.2%, and 13.9% and had been held in metal containers for 9 years at 21°C and 1 year at 12°C. In order to simulate storage in sacks, the samples had been aerated weekly and at the end of the first three years of storage their moisture contents had shifted somewhat to the levels shown in Table 1. The table also gives the final levels of thiamine, niacin, and riboflavin in the raw beans.

No proper control sample was available. However, USDA Handbook Nº 8 (25) gives the following average values for raw dry pinto beans: moisture, 8.3%; thiamine, 0.84 mg/100 g; niacin, 2.2 mg/100 g; riboflavin, 0.21 mg/100 g.

It is apparent that there was a very substantial loss of thiamine during storage and that it was progressively greater at the higher moisture contents. Niacin seems to have been retained well at all moisture levals. While it would be tempting to conclude that there had been an increase in riboflavin during storage, as Chitre *et al.* observed in their work, the precision of our determinations does not justify such a conclusion.

Germination tests on the three samples showed essentially 100% viability in Sample A and 0% in Samples B and C.

Decoctions in distilled water gave the following pH values: A, 6.53; B, 6.30; C, 6.27.

There was also the question whether there had been a change in the quality or availability of the protein in the stored beans. Two different effects might be observed. First, there might be a loss of methionine-cystine and/or of lysine during prolonged storage. Secondly, the increase in required cooking time resulting from high-moisture storage would decrease the protein value further.

The very limited size of the samples made it possible to conduct only one simple experiment, with Samples A and C. Required cooking times (after presoaking) were determined subjectively to be about 80 minutes for the former and 590 minutes for the latter. Three samples were then prepared: A-80 (Sample A cooked 80 minutes); A-590 (Sample A cooked 590 minutes);

TABLE 1
VITAMIN CONTENTS OF RAW PINTO BEANS STORED 9 YEARS AT 21°C

Sample	Moisture Content	Thiamine		Niacin[3]	Riboflavin[4]
		Chemical[1]	Microbiol.[2]		
	%	mg/100 g	mg/100 g	mg/100 g	mg/100 g
A	8.6	0.59	0.66	1.58	0.22
B	12.2	.14	.16	1.64	.30
C	13.5	.08	.10	1.54	.32

1/ Thiochrome method (21)

2/ L. fermenti method (22)

3/ L. arabinosus method (23)

4/ L. casei method (24)

TABLE 2
PROTEIN EFFICIENCY RATIOS AND DIGESTIBILITIES AFTER 9 YEARS STORAGE AT 21°C

Sample	PER[1]	Nitrogen Digestibility %
A-80[2]	1.19	76
A-590[3]	0.87	71
C-590[4]	0.96	72

1/ Corrected to casein = 2.50

2/ Stored at 8.6% moisture, boiled 80 minutes

3/ Stored at 8.6% moisture, boiled 590 minutes

4/ Stored at 13.5% moisture, boiled 590 minutes

and C-590 (Sample C cooked 590 minutes). These were freeze-dried and each was fed to a group of 10 rats at a level to supply 10% protein in an otherwise balanced diet. After 4 weeks Protein Efficiency Ratios (PER) and percent nitrogen digestibilities were calculated. The results are shown in Table 2.

Sample A-80 was significantly higher in PER and in nitrogen digestibility than the other two samples ($P = 0.01$); the latter two were not significantly different from each other ($P = 0.05$). Taken at face value, this would mean that no difference could be attributed directly to storage but the longer cooking necessitated by storage at the higher moisture level did cause a significant loss in both PER and digestibility.

Nutrients Destroyed by Pests

So far we have considered only those losses of nutrients which occur through chemical or enzymatic reactions taking place within the legume seeds themselves. However, nutrients are lost just as surely during storage if the seeds are destroyed by rodents, insects, or microorganisms. The U. S. President's Science Advisory Comittee (26) has reviewed the few experimental and survey data and various estimates of the magnitude of such losses that have been published. The numbers are impressive and shocking. For example, an FAO survey report which is cited shows that a minimum of 30% of the pulses produced in India were lost during storage. In Uganda, beans stored for 6 months by the producer showed 38-69% of the seeds damaged by insects alone. In Northern Rhodesia 12 months producer storage of beans and of cowpeas *(Vigna unguiculata)* resulted in over 80% insect demage. In 1946 an FAO expert committee estimated that 10% of all cereals, pulses, and oilseeds produced throughout the world are lost during storage.

SUMMARY

"Hardshell", the reluctance of certain beans to imbibe water and swell, is reversible. The percentage of hardshell beans in a given lot increases during storage in air of low relative humidity but will decrease if they are transferred to moister air. As a practical matter, hardshell can be overcome by boiling or steaming beans for a minute or two at the beginning of the soaking period. An entirely different phenomenon is a progressive loss of cookability, i.e., an increase in the length of time required for presoaked beans to cook tender. This change is promoted by high moisture content and high temperature during storage. At least a few-fold increase in required cooking time may occur if beans are held for a year even at 21°C and a moisture content below the maximum of

18% permited by the U.S. Standards for beans. The limited data in the literature indicate that the biological value and digestibility of the protein of beans decline during storage, presumably through loss of sulfur-containing amino acids and/or lysine. There is also a loss of thiamine but niacin and riboflavin are well retained. New data are presented that show that the rate of thiamine loss is greater when beans are stored at a moderate moisture level than at a low level and that the longer cooking necessitated by unfavorable storage conditions causes a drop in Protein Efficiency Ratio and in nitrogen digestibility. In addition to these losses in nutrients through changes in beans themselves, substantial losses are caused by microorganisms, insects, and rodents when storage facilities are inadequate.

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Changes in chemical composition and nutritive value of common beans and other legumes during house cooking

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INTRODUCTION

Beans are an important source of protein and calories in the diet of many countries in Latin America (1-3).

Nutritional and dietetic surveys in Central America on the consumption of beans (4-10), have indicated that after corn, beans are the most important food in the diet of many rural groups.

In general, beans are consumed in Central America in different ways: whole-cooked, whole-fried, and strained and fried, and these rutinary preparations require different degrees of heating, which could damage the nutritional quality of its protein.

Raw legume grains are in general characterized by the presence of toxic factors (11, 12), which when fed to animals, affect growth adversely and increase mortality. Furthermore, legume seeds show a low digestibility, not only of the proteins, which results in a low absorption of amino acids and nitrogen, but also of fats. Besides, a decrease in food intake —the factors responsible for this state— could be the toxic compounds present in legume grains which in most cases, are destroyed by heating (11).

This effect has been well reviewed and it is well known that the nutritive value of legume foods is greatly improved by the use of heat (11, 13-15). The degree of improvement in nutritional value accomplished by heat is dependent on the temperature and

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heating time used, and on the moisture conditions of the sample (15) In the various studies carried out in this area, the type of cooking treatment used varies significantly from one author to the other, and therefore, it is very difficult to have uniform conditions for comparison purposes. Thus many authors agree that prior to cooking, it is advisable and recommendable to submit legume grains to a soaking process (15-19). This is due, in the first place because this treatment does not induce any loss of nutrients (16), and second, during the soaking process many toxic substances are probably lost, specially when the water used for the soaking is discarded (16, 18). Moreover, it causes a certain softening of the seeds and therefore, cooking conditions are less drastic. There is a slight variation regarding the length of soaking periods recommended but the practice most often used has been to let the legume grain soak over-night (16, 18).

With respect to cooking periods, Bressani *et al* (20) have recommended autoclaving at 16 lb pressure and 121°C for 10 to 30 minutes. Heating for longer periods results in a decrease in the nutritive value of the protein, due to changes in the essential amino acid content of the bean, specially in the content of available lysine, which decreases proportionally to the increases in cooking time. It is important to point out that home-cooking conditions (four hours) have the same effect as autoclaving, due to the lower pressure and temperature utilized in the former. However, it has been reported that home cooking does n t necessarily destroy all antiphysiological factors, particularly hemagglutin activity.

Kakade and Evans (19), autoclaved beans at 121°C, during 5 minutes and when these cooked samples were fed to rats, the animals gained weight and did not die. There are some results that indicated that higher temperatures during the same length of time, do not promote as much growth due to destruction or inactivation of some essential amino acids in the over-heated beans.

Liener (21), studying the effect of cooking noted that when beans were autoclaved at 121°C, during 30 minutes, the weight gain of chicks improved markedly in comparison to those animals fed the raw beans. Besides, feeding the latter resulted in an enlargement of the pancreas, effect which was eliminated by cooking the diet.

Braham *et al* (12) found that the optimum cooking temperature for pigeon peas *(Cajanus cajan)* was 20 minutes at 121°C and 16 lb pressure, and a longer heating time lowered the nutritive value of the beans.

Jaffé (18), has recommended autoclaving the seed after a soaking period of 30 minutes, observing that under these conditions the main toxic effects —specially in black beans and Hyacinth— are destroyed; the toxic effects however, are not removed if the material is autoclaved in a dry state, or without a previous soaking period. The cooking period does not affect the toxic factors alone, since it was already mentioned that it also decreases the availability of some amino acids such as lysine and cystine (16). Jaffé (22) observed that legume seeds with a higher trypsin inhibitor activity are also those whose *in vivo* digestibility improved after being cooked. This is probably due to the fact that trypsin inhibitors are rich in cystine and when they are inactivated by a cooking process, more sulphur amino acids become available (18, 23). On the other hand, cowpeas *(Vigna sinensis)* may be added to vegetable mixtures (either cooked or raw), without any toxic effects. Supplementation with methionine, increased the nutritive value of both, cooked and raw cowpeas, but the difference between the two was not significant (24).

The cooking treatment may also affect the vitamin content of legume seeds. For example it is known that it causes a decrease in thiamine (25), specially under two conditions, when they are placed in boiling water in order to produce a fater softening (16), and when they are cooked with bicarbonate (26), for the same purpose.

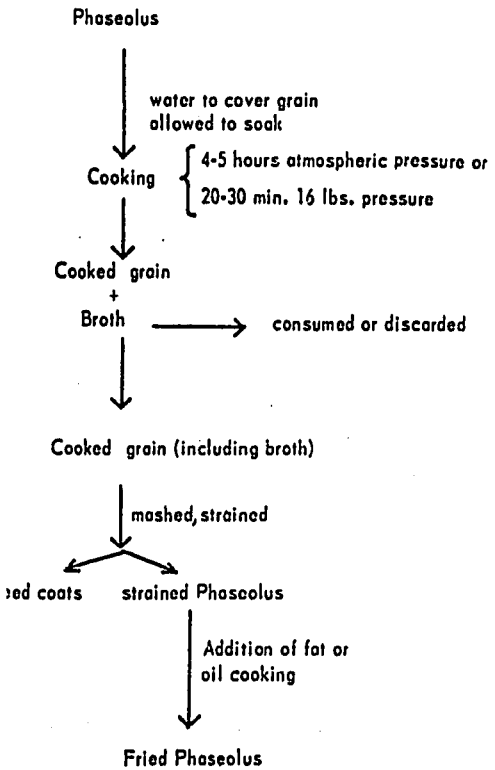
The above review suggests a lack of knowledge on optimum bean cooking conditions, both at the home and at the laboratory level. The results to be presented will serve to stress the fact that many other aspects must be considered to be able to explain the chemical and nutritional changes taking place during bean cooking. The first part describes the changes taking place at the home level, and the second, presents data on the effect of other variables not always considered in nutritional evaluation of beans. From the results to be shown a series of recommendations for future research will be presented.

MATERIALS AND METHODS

Cooking Process

The black beans used in this study were obtained from INCAP's experimental farm, and were considered of as good a quality as those ordinarly consumed in rural and urban areas of Guatemala. The home cooking process used in these experiments is shown in Graph

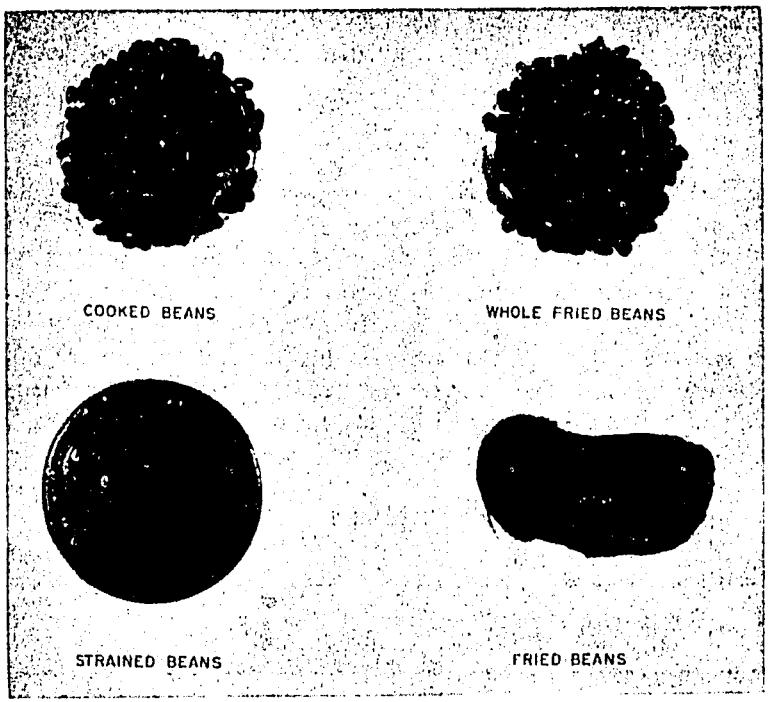
No. 1. As can be seen the grains are placed in sufficient water to cover them completely. An additional amount of water, about 4 liters of water/kg of beans, is then added to compensate for the absorption and evaporation which takes place during the cooking process. Onions and other flavoring ingredients are usually added and the whole preparation is then cooked at atmospheric pressure for 4 to 5 hours, or 20 to 30 minutes in a pressure cooker, until they are soft. Once softness is accomplished, the bean broth, if available, is consumed as a soup, with rice, cubes of toasted bread or other such foods. The cooked beans may then be consumed as such, or fried with vegetable shortening, usually accompanied by plantains, rice, or corn in the form of tortilla. By mashing and straining the cooked beans, including the broth, the seed coat is separated and strained beans are prepared. These are then mixed with fat to make fried beans. There are variations to the above cooking process, particularly with respect to the addition of salts,



Graph 1. Home cooking process.

such as sodium chloride and sodium bicarbonate. However, in most countries, particularly in urban areas, fried beans are most popular among the consumers.

Phohograph No. 1. shows the physical aspects of the preparations above mentioned, cooked, whole-fried, strained and fried beans.



Photograph 1. Physical aspects of different preparations of beans.

Chemical Analysis

Proximate chemical composition of the samples was determined using the methods of the O.A.A.C. (27); available lysine was analyzed according to the method of Conkerton and Frampton (28); methionine by microbiological assay using the method of Steele *et al* (29); iron, by the method of Moss and Mellon (30), and phosphorus by the techniques of Fiske and Subbarow (31).

Experiments with rats

The rats used were from the Wistar strain of INCAP's colony and the diets used in all growth experiments were made by using

the different dry bean preparations as the only source of protein, to provide a 10% level in the diets. The diets consisted of (%): Mineral mixture (32) 4.0, cottonseed oil 5.0, cod liver oil 1.0 and sufficient corn starch to adjust to 100 grams. All diets were supplemented with 5 ml of a vitamin solution (33).

PER. (Protein Efficiency Ratio) - was carried out according to the common technique utilized at INCAP for many years, which is essentially that of the A.O.A.C. (27).

Nitrogen Balance. Nitrogen Balance was carried out as described elsewhere (34).

RESULTS AND DISCUSSION

The results of calculations of recoveries or material balance using the home process of beans are presented in Table 1. As can be seen, the recoveries for the three main preparations were 85.6%, for cooked beans, 61.6% for strained beans, and 93.6% for fried beans, calculated from the raw material on a dry basis. The recovery of the fried beans is higher due to the fat added necessary for its preparation. The recovery of strained beans is lower probably due to losses that occur during its preparation. The residue is equivalent to 11.8% of the raw bean weight.

TABLE 1
BALANCE OF MATERIALS

	Weight of sample g.	Water content %	Total water g.	Dried weight g.	Recovery %
Raw beans	1900	14.1	268	1632	100%
Cooked beans	3744	62.7	2347	1397	85.6%
Strained beans	3240	69.0	2236	1004	61.6%
Residue	580	66.7	387	193	11.8%
Fried beans	4228	52.4	2215	2013*	93.6%

* Dry weight with fat added.

The changes in chemical composition are shown in Table 2. As can be seen, the moisture of the preparations were very similar in both whole-cooked beans and strained beans including the residue. On a dry weight basis, nitrogen and fat content were also similar in these two preparations. Regarding the ash content on the dry weight basis, there is little variation among whole-cooked, strained and fried beans, but the residue has a high percentage of ash and also

of crude fiber. Beans fried with vegetable shortening showed a lower protein content due to the fat present which at the same time raises its ether-extract content.

TABLE 2

PROXIMATE CHEMICAL COMPOSITION OF RAW BEANS AND PREPARATIONS

(In 100 g sample)

Home preparations	Raw Beans	Cooked Beans	Strained Beans	Fried Beans	Residue
Moisture of preparations, g	14.1	62.7	69.0	52.4	66.7
Moisture of dried sample, g	4.09	4.41	7.07	--	4.64
Ether extract, g	1.9	0.7	0.6	13.3	0.6
Crude fiber, g	6.4	2.8	1.6	1.6	13.3
Protein, g	24.56	24.78	24.00	17.79	20.88
Nitrogen, g	3.93	3.97	3.84	2.85	3.24
Ash, g	3.43	2.69	2.13	2.28	4.39
Carbohydrates, g	59.62	64.62	64.60	--	56.19
Iron, mg	9.71	7.31	9.39	4.00	10.42
Phosphorus, mg	306	--	340	229	281
Calcium, mg	336	218	377	322	118

The changes in nutritive value of these bean preparations are shown in Table 3. As can be seen, cooking results in an improvement of PER from o for raw beans, to 1.24 and 1.43 for cooked and strained beans respectively, due to the destruction of antinutritional and toxic factors. Similar results have been reported by other investigators (11, 35-37). Fried beans show a slightly lower value probably due to the more drastic thermic treatment suffered, which can cause the destruction of some essential amino acids, such as lysine and/or methionine (38). Of interest is the relatively high PER value of the residue, suggesting it includes seed componentes besides the seed coat.

The results of biological assays with rats, using nitrogen balance in order to determine protein digestibility and biological value of the beans preparations, are shown in Table 4. As can be seen,

TABLE 3
GROWTH OF RATS* FED BEANS PREPARED IN DIFFERENT WAYS

Home cooking	Protein %	Average weight gain	Protein Efficiency Ratio**
Raw Beans	10.19	--	--
Cooked Beans	10.50	34	1.24
Strained Beans	10.25	37	1.43
Fried Beans	7.69	10	0.87
Residue	9.94	25	0.99
Casein	10.81	130	2.73

* 8 rats/group. Average body weight: 50 g.
** PER: weight gain/protein consumed.

nitrogen balance was slightly lower for fried beans, protein digestibility was similar among the preparations and the biological value was a little higher for fried beans probably due to the caloric content of this preparation. The apparent discrepancy between PER and Biological Values obtained for the fried beans, is due to the lower protein content in the diet made with this preparation for the PER assay. As is well known in the case of Nitrogen Balance, protein intake is kept constant, however, in the PER assay protein level was not a limiting factor because animals consumed *ad libitum.*

TABLE 4
BIOLOGICAL VALUE OF COOKED, STRAINED AND FRIED BEAN PREPARATIONS*

	Nitrogen Balance, g	True Protein Digestibility %	Biological value
Cooked beans	0.184 ± 0.04	67.9 ± 6.3	65.5 ± 7.4
Strained Beans	0.186 ± 0.02	71.7 ± 2.9	62.5 ± 9.2
Fried Beans	0.172 ± 0.05	69.1 ± 5.1	66.1 ± 7.2

* Diet intake g/rat: 8, Average: 7 rats.
Diets containing 10% bean protein.
Cooked without soaking.

The effect of cooking time on the protein quality of beans *Phaseolus vulgaris)*, is presented in Table 5. For this experiment ıe beans were cooked in an autoclave from 0 to 180 minutes ithout a previous soaking period. The results show that protein uality is affected after 30 minutes of heating demonstrated by decrease in PER and in the available lysine content as the ooking time increases.

These results show again that cooking time, particularly heating eyond 30 minutes, results in a decrease in the amounts of available sine due to the interaction of this amino acid and carbohydrates 39). It is possible that there is a relationship between cooking me and cooking temperature, relationship which should be otained with specific and well known bean varieties and species. owever, these are not the only variables of significance as will e shown.

TABLE 5
FFECT OF COOKING TIME ON THE PROTEIN QUALITY OF BEANS

Cooking period minutes*	Weight gain g/28 days	Protein Efficiency Ratio	Lysine Available g/16 g N
0	0**	0	7.96
10	75	1.31	6.13
20	72	1.35	5.10
30	76	1.29	5.79
40	59	1.20	6.28
60	35	0.89	--
90	37	0.92	--
120	37	0.88	--
150	29	0.73	--
180	24	0.63	--

* Cooked in the autoclave at 121°C and 16 lbs pressure.
** All animals died.

The previous results were obtained with dry beans stored for months. Since storage can affect the cooking characteristics of gume foods, more detailed studies were carried out in samples recently harvested beans. Table 6 shows the effect of cooking ese beans at different periods of time, and of soaking.

TABLE 6

RELATION BETWEEN PROTEIN EFFICIENCY RATIO (PER), DIGESTIBILITY AND LYSINE AND METHIONINE CONTENT OF BEANS* UNDER DIFFERENT THERMAL TREATMENTS

rocess**	P.E.R.	Digestibility in vivo %	Digestibility in vitro %	Available Lysine g/16 g N	Methionin g/16 g N
0 - 10	1.47	80.2	84.4	4.98	0.619
0 - 20	1.26	78.5	82.6	4.20	0.566
0 - 30	1.11	75.5	81.0	4.73	0.686
8 - 10	1.52	79.9	84.1	5.23	1.496
8 - 20	1.13	78.9	82.9	5.28	0.878
8 - 30	0.96	77.1	81.3	4.31	0.778
16 - 10	1.36	80.8	82.6	5.00	0.758
16 - 20	1.09	76.6	81.6	4.43	0.686
16 - 30	0.94	79.1	79.7	4.31	0.648
24 - 10	1.43	77.8	81.1	4.69	0.579
24 - 20	1.16	77.6	79.9	4.98	0.693
24 - 30	1.11	76.6	78.7	4.33	0.763

Soon after harvest.

* Soaking, hours - cooking, minutes.

TABLE 7

NEEDS FOR FUTURE RESEARCH ON LEGUME SEEDS

1. Chemical and biological evaluation of the interrelationship between soaking and cooking times.
2. Use of additives (salt, garlic, onions).
3. Effect of storage on the cooking characteristics of dry beans.
4. Identification of toxic factors in raw and cooked common beans and in other species and varieties of legume seeds.
5. Responsible factors for the low protein digestibility in legume foods.
6. Standarized methodology for the selection of genetically improved legume seeds.

The results indicate that to obtain a greater nutritive value, the optimum cooking time found was 10 minutes in samples subjected to soaking periods of 0, 8, 16 and 24 hours. In the treatments studied, even though no significant decrease was observed in the available lysine and methionine levels, there was a decrease in nutritive value which could be related to a lower protein digestibility as shown by the figures for *in vivo* and *in vitro* protein digestibility.

The graphic representation of these results can be observed in Figure 1. The results indicate that in the case of fresh, recently harvested beans, the PER value is affected by a cooking time in excess of 10 minutes even in samples not subjected to soaking. Moreover, these results also suggest that in the case of recently harvested beans, the hydration of the seeds took place easily and quickly in the cooking water. This indicates that the thermic damage observed after 20 minutes of cooking, also occurs in samples not subjected to a previous soaking period. According to these results, recently harvested beans need shorter cooking and soaking times than stored beans. These aspects are very important from both the standarization of the soaking methodology and from the industrial point of view as well.

In the next experiment it was observed that during dehydration of the cooked beans the seeds developed a tendency to adhere probably due to a high sugar content in the beans recently harvested.

It was interesting to determine how the available lysine content of the seeds changes in samples subjected to different heat treatments. The results are presented in Figure 2.

These results indicate that increasing the cooking time produces a decrease in available lysine in all heat treatments tested. Even though the changes in available lysine do not exhibit a statistical relation with the observed changes in PER, it is assumed that the decrease in available lysine content due to increasing cooking times would result in a biological dagame to the protein.

The last Table points out some of the research areas where there is need for further investigations. It is obvious that more research should be carried out on point Nº 1 because of the interrelationship previously found between the effect of soaking and cooking time and to items Nº 2 and 3, since relatively very few studies have been carried out either on the use of additives or on the effect of storage on the cooking quality of dry beans.

The results obtained will be useful in standarizing the cooking methodology as well as the industrial processing of beans.

Further studies on points Nº 4 and 5 should help in better understanding the effect of toxic factors as well as the low protein digestibility on the nutritive value of beans.

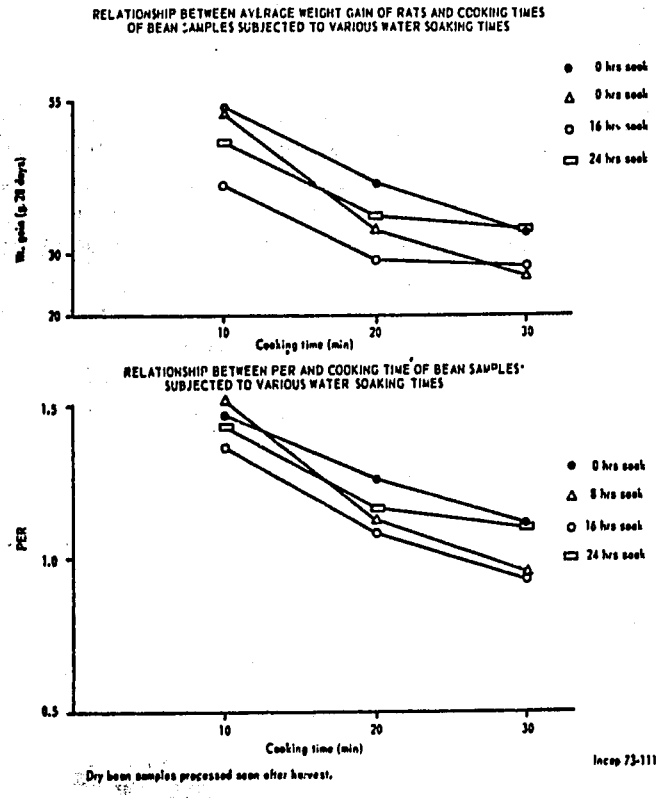


Figure 1. **(top) Relationship between average weight gain of rats and cooking times of bean samples subjected to various water soaking times.**
(bottom) Relationship between PER and cooking time of samples subjected to various water soaking times.

Finally, research on the last point is needed to help geneticists select nutritionally improved legume seeds. The methodology along these lines should include chemical as well as biological techniques. There is need also to develop methods to assess the effect of sample preparation and processing conditions on the nutritive value and on the presence of toxic constituents that are resistant to the thermic treatment used in different beans preparations.

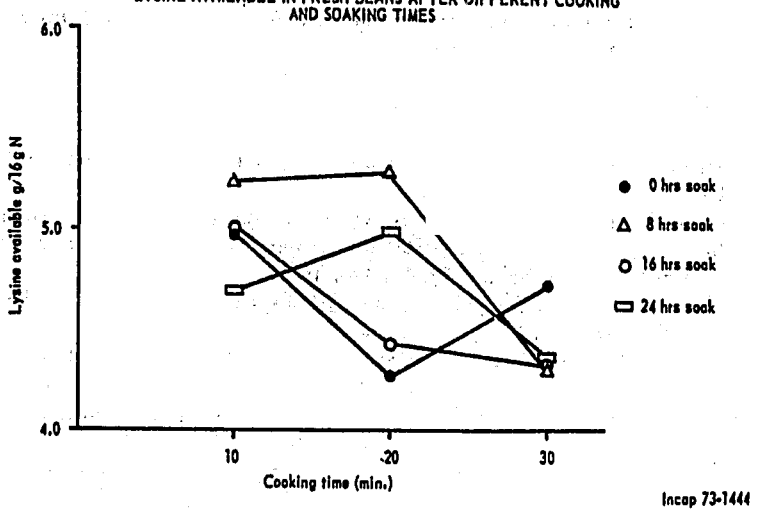


Figure 2. Available lysine in fresh beans after different cooking and soaking times.

SUMMARY

Home processing of beans (Phaseolus vulgaris) in Central America includes water soaking and cooking in a pressure-cooker or at atmospheric pressure, and preparation of whole-fried or strained and fried beans. The proximate chemical analysis of these home preparations shows that the moisture, nitrogen, and fat content was very similar in both whole cooked beans and strained beans, and its residuum. In regard to ash, there is little variation among whole-cooked, strained and fried beans, but the residuum has a high percentage of ash and crude fiber. Beans fried with vegetable shortening showed a lower protein content due to the fat present which, at the same time, raises its ether-extract content. Biological assays in rats indicated that, of the preparations studied, the nutritive value was more affected in the case of fried beans, followed by strained beans, and whole-cooked beans. In the case of fried beans, the greater decrease in protein quality was brought about by the more drastic thermic treatment suffered in comparison with the other preparations. More detailed studies on whole-cooked beans indicated that to obtain a greater nutritive value, the optimum cooking time found was 10 minutes in samples subjected to soaking periods of 0, 8, 16 and 24 hors. In the treatments studied, even though no significant lowering was observed in the available lysine and methionine levels there was a decrease in nutritive values which could be related to a lower protein digestibility; this aspect should be further studied. The results also indicate that it is necessary to evaluate chemically and biologically the interrelationships between soaking and cooking time, as well as salt addition, on the nutritive value and toxic factors in common beans and different grain legume and species

varieties. Similar studies should be carried out in stored beans. These parameter could be utilized for the purposes of improving genetically selected materials through a standardized methodology.

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Preparation and utilization of dry, canned, and precooked beans

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INTRODUCTION

The importance of dry beans, or legumes in general, as human food derives from a fortunate coincidence of at least three major factors: their low cost, a relatively well-balanced food value, and ready acceptance in many cultures. We in the U.S. have been popularizing a concept of food as composed of four categories, including meats, dairy products, fruits and vegetables and cereals. Perhaps the pulses merit consideration as a fifth.

The diversity of pulses is nowhere more apparent than here in Latin America. Having worked with several of the varieties of beans marketed in the U.S., and some from Africa as well, my professional curiosity is aroused by the different types that I see in the market place, both here in Brazil and in Mexico where I visited last year. They may represent an even wider range of attributes, as affecting processing, than I have already experienced.

A great deal of research attention has already been given to this food resource. Witness the long series of Dry Bean Research Conferences held in the U.S., the latest, or 11th of which is convened in Rochester, NY on this very day (an unfortunate coincidence for me). But I think that our presence here in an international gathering is proof of a heightened interest in improving one of the basic foodstuffs. We hope that as a result the pulses will eventually contribute an even greater share of the nutritional sufficiency that we are seeking for all the world's peoples.

The discussion to follow will be confined to a consideration of dry beans, though many of the principles will apply to other legumes in one way or another. In this paper we shall not present any new data but rather will attempt to present an analysis of what is required in bean processing and utilization. This may clarify what

we already know and serve as a point of departure for seeking new information toward the solution of unsolved problems in processing.

There are three major forms in which dry beans are offered and utilized: the naturally dried, uncooked beans, the ready-cooked ("canned") product preserved in a hermetically - sealed metal container, and the quick-cooking or precooked, dehydrated forms. Dried bean powder will be considered elsewhere, and so the discussion will also be limited to more or less the whole bean product.

The step-by-step preparation of beans for the table should first be outlined in order to highlight the differences and show the similarities among these product types. Processed beans will of necessity have been hydrated to a microbiologically unstable level of water activity in order to be properly cooked. Therefore, those products that may be variously described as quick-cooking, precooked, or merely reheat-and-serveable will require extra steps in preservation. This may involve cooking, drying, and packaging that would not have to follow if consumption were immediate.

Preparation, or processing, may be broken down into the following operations:

1. cleaning and sorting;
2. hydration of the naturally dry beans,
   which may be preceded by or include a blanch;
3. cooking of the hydrated bean;
4. preservation treatment by canning, drying, or freezing, plus cooling from the cook temperature;
5. packaging,
   to maintain the microbiologically stable condition provided by the preservative method;
6. storage and distribution,
   as related to preservative method and packaging; and
7. final preparation for the table,
   whether in the home or in a dining establishment.

We might assume here for the sake of simplicity that the beans are both clean of field dirt and free of extraneous material, though a processor would have to provide for these operations. Then it remains to improve both the palatability and digestibility of the beans so that they may be readily eaten and efficiently utilized in the body. Those requirements are met by thermal treatment, or simply cooking, a procedure optional in many foods, but defi-

nitely required for dry beans. While it is obviously true that for greater ease of ingestion, raw beans could be broken into fragments or ground into a powder, digestion would undoubtedly be a slow, painful, and incomplete process. In fact, the presence of anti-nutritional factors, such as trypsin inhibitor and hemagglutinins, that interfere with protein utilization might in that case lead to sickness rather than nourishment. Some degree of thermal treatment is necessary to destroy such factors and to provide the predigestive changes that will insure efficient digestion in the body. In general terms, these preliminary changes may be identified as pregelatinization, or even hydrolysis, of the carbohydrates and denaturation of the protein. Both are greatly accelerated, beyond what would occur to raw material in the digestive tract, by heating to temperatures in excess of 85°C in the presence of water.

Hydration

It is apparent that hydrolysis will require intimate contact of the bean material with water on a cellular level. While more rapid contact might be achieved by first grinding the raw bean to a powder, we are confining ourselves to consideration of the whole bean, or at least intact cotyledons. The timehonored method has been simply to immerse the beans in water, the required soak period depending on the temperature maintained and on the permeability of the seedcoat. Dry beans, starting at a typical moisture level of 12-14%, take up enough water during hydration to increase their weight by a factor of about 2. The rate of uptake is first rapid - then slower, and it is reasonable to inquire at what exact degree of hydration the succeeding step of cooking might be initiated in order that the hydration period be kept as short as possible.

Unfortunately, this approach is foiled by the fact that water uptake is not uniform, either among the individual beans or within a bean. What must be dealt with is not uniformly incomplete hydration but some beans, or some spots in individual beans, which have not hydrated sufficiently. This condition would result in some incompletely cooked beans, unless enough time is allowed for more complete hydration or the slow-hydrating units are removed.

Suggestions for the separation of incompletely hydrated beans have ranged from screenng for size or density to differentiating the elastic properties, or bounce. Screcning out small, supposedly unhydrated beans would only be satisfactory if the dry beans were size-

graded beforehand, as otherwise fully hydrated but undersize beans would be unnecessarily removed. However, processing separate fractions of uniform size beans might offer advantages in the specification and close control of hydration, cook, and drying times and result in superior product appearance. Therefore, size-grading might be a useful preliminary to thermal processing. The specific gravity of beans varies in a predictable pattern, during hydration, probably depending on variety, and this might be made the basis of a separation technique. Utilizing elastic properties to separate beans that respond slowly to hydration and therefore retain greater bounce requires more imagination and engineering ("imagineerin"?), but at least invites study.

Various treatments have been used to accelerate hydration. The most obvious of these is elevating the soak temperature, since the beans must eventually be heated for cooking anyway. Dawson et al. (1) suggested a quick hydration that commenced with a short (2-min) boiling treatment followed by slow cooling in ambient air over the subsequent one-hr period. In our laboratory we have found that for the pea or navy bean an overnight soak of 16 hr at 20°C is reduced to 4-½ hr at 40°C, and 40 min at 82°C. However, even the last is too long to be readily achieved in continuous equipment, except perhaps by operating two reel-type blanchers in series. And 82°C is about as warm a soak as can be used without getting partial cooking and excessive softening of texture at the same time.

Superimposing other methods for hastening hydration on the high-temperature soak might shorten the required time still more. For example, Hoff and Nelson (3) found that the release of adsorbed or entrapped gases from the bean surfaces, by means of steaming or the application of vacuum or sonic energy, accelerated hydration. This degassing, together with the inclusion of polyphosphates and salt in the soak water, readied the beans for cooking in 20 min at 80°C. Even with this multi-faced approach, it may remain to deal effectively with a small proportion of "hard-shell", slow-hydrating beans, such as by removing them, to prevent their adverse effect on the final product.

The Hydravac process, in which lima or other dry beans were subjected to intermittent vacuum treatment over a 30 - to 60 - min period in a solution of inorganic salts and then soaked for an additional 6 hr in the same solution to provide a quick-cooking dry bean, was contributed by Rockland and Metzler (9). The salts

used included sodium chloride, sodium tripolyphosphate, sodium bicarbonate, and sodium carbonate.

Presteaming the product has been claimed by Dorsey et al. (2), Steinkraus et al. (10), and Morris et al. (8) to have beneficial effects on the subsequent rate of hydration as well as accomplishing destruction of lipoxidase. LaBelle (6) found that a few minute's presoaking in acetone or ethanol shortened hydration time dramatically, though this may be mainly of academic interest. Similar treatments with acid or alkali proved ineffective. These treatments with steam, inorganic salts, or solvents probably modify the special characteristics of the seedcoat that render it relatively less permeable to water than the cotyledons. Implication of the seedcoat as a barrier is borne out by observation that scarifying it more rapidly initiates hydration at that point and by the work of Kon et al (4) in mechanically peeling and splitting beans to achieve faster cooking, whether soaked or unsoaked.

Cooking

We have delt at length on hydration because it is a necessary preliminary to cooking. In the preparation of a precooked, dehydrated product, the temporary introduction of moisture, only to remove it again by redrying the beans when precooking has been accomplished, is an expensive necessity. But the desired cooking effects have not been achieved any other way. Application of dry heat to the unsoaked dry bean, which might be referred to as toasting or parching, apparently has very different effects on flavor, appearance, texture, and the digestibility of the nutrients, though little has been reported on this alternative.

Once a sufficient level of hydration has been attained, cooking is simply done. In the pea bean the imbition of nearly an equal weight of water to arrive at a moisture content of about 55%, is the necessary preliminary. The extent of cooking, as measured by any one of its principal effects, is related to the time-temperature product, but these relationships have not been well documented. LaBelle et al (5) showed the combined effects of cooking time and the level of sugar coating on both surface and interior color, texture, extent of butterflying, rehydration ratio, and PER in the preparation of precooked, dehydrated pea beans, but only one cook temperature (121°C) was used. Data previously obtained on cooking at other temperatures in the range 210-260° F (100-127°C) was exploratory and incomplete.

The same approach could be extended to a complete matrix of cooking times and temperatures within a range of practical, or even academic, interest to show the relationships of the time-temperature product to the various cooking effects. These would include the pregelatinization and partial hydrolysis of the starch fraction and the denaturation of the protein as related to digestibility, and the softening of texture as related to ease of ingestion. Changes in color and flavor would be considered incidental, though obviously important in marketability. When superimposed on a diversity of bean types and hydration regimes, the task of obtaining such a large body of data becomes very great and expensive. Hewever, it would contribute much to our still incomplete understanding of physical and chemical changes involved in the combined process of bean hydration and cook.

There are no doubt porcesses used in the preparation of canned beans in which there is no clear-cut separation between hydration and cook. Dry beans can be filled directly into the can, syruped with whatever packing medium is desired, and then subjected to a long slow heating. Hydration is accomplished during the early, low-temperature part of the heating cycle, slowly merging into a cooking effect as the final process temperature, probably in excess of 110°C, is reached and maintained. Beans packed in heavy sauce in No. 303 cans (16.88-oz., or 480-g capacity) and filled at 100°F (38°C) require a cook of 95 min. at 240°F (116°C) (7), so that there is time to accomplish both hydration and cook. A similar process has been attempted by the authors in the preparation of precooked beans, with the hydration water drained off as a programmed temperature rise from 180 to 250°F (82 - 121°C) proceeded through 210°F (99°C). Failure to drain the water off soon enough led to mushy beans unsuitable for subsequent dehydration.

Preservation and Packaging

Preservation and packaging are interrelated in that the mode of preservation defines the package type. Preservation in hermetically-sealed cans follows naturally from a process that calls for a pressure cook in the same range of time and temperature as demanded by the requirements for commercial sterility of the pack. An exact coincidence would of course depend on bean type and can size, but hydration conditions before filling and the fill temperature itself can be modified to improve the fit. At least, the specified retort process is much more appropriate to the required cooking

effect than in most fresh vegetables and fruits, where excessive or undesirable changes in color and texture may occur. Both the original dry bean and the precooked, dehydrated product, on the other hand, are microbiologically stable at moisture levels variously in the range 8-14% and can be symply economically packaged in cartons or bags.

Storage and Distribution

In the matter of the storage and distribution of these products, both package types can claim advantages. The sealed metal can affords better protection against accidental damage, insect infestation, or changes in moisture content. However, in balance the can remains at some disadvantage because of the waste space (22%), entailed in packing cylindrical containers in rectangular cases, and even more so because of the expense of packaging, storing, and handling the water of hydration plus that in the packing medium - taken together about tripling the weight of the product.

Utilization

If the canned product is handicapped in storage and distribution, it recoups somewhat in its convenience of use. It needs only to be heated and served, or can even be consumed without heating. In the U.S. a greater variety of formulations are available than ever before, with various sauces - tomato, molasses, and barbecue, and in combinations with several meats, such as pork, franks, and beef. This increasing variety in part offsets the claim made for either the raw, dry bean or precooked or quick-cooking products that they offer greater flexibility in recipe by permitting the user to prepare them in some favorite, traditional, or regional way. However, any of the canned or pre-processed dry products enjoy a distinct advantage in the consumer's eyes over the raw, dry bean because of its long preparation time and heavy demands for fuel. While it is difficult to generalize, it is almost certain that the overall manual effort and fuel requirements are less for preparing the product in large quantities in the food processing plant than in meal-size quantities in the home. This is despite the extras entailed in heating twice, as in the case of the canned product, or of heating twice plus drying, in the case of the precooked beans. Whether it is safe to say that the total energy requirements are less, considering the extra expenses of packaging, storage, and handling in the case of the canned product, is something else again.

Nutritional Aspects

A first consideration is with the extraction and possible loss of water-soluble vitamins and other nutrients in the soak water, if it is discarded. This could occur either in the factory or in the home. However, it is not clear whether this effect is greater for a short rehydration period at elevated temperature than for the much longer time required when soaking at ambient. The loss can be avoided if the soak is used as the packing medium for the canned product, and if the liquid or sauce is subsequently consumed rather than wasted. Whenever the loss is significant, this would represent one disadvantage in the preparation of the precooked, dehydrated product where the soak water is not retained. Reuse of soak water in an effort to reduce losses would require special management to avoid microbial spoilage.

What may be more important is the possible destruction of heat-sensitive nutrients such as certain vitamins and amino acids during the cook. Again, a time-temperature product is involved, just as it is in the attainment of desirable cooking effects. A sufficient process in the latter sense may perhaps be obtained at any temperature in the range 100-150 C, if the cooking time is correspondingly long or short. But as pointed out before, we do not yet know enough about the process to give a formula for this time-temperature product based on desired cooking effects, whether considered individually or together. The cook time chosen for a particular temperature would surely depend on the type of bean and on the particular cooking effect given prime consideration.

TABLE 1

Reduction in PER in pea beans steam-cooked at 121°C.

Cook time, min.	Ajusted PER*	
	without dextrose	with dextrose
10	1.18	.17
20	1.03	—.15
40	.77	—.16
80	.72	—.52

* PER of casein control was 2.72; data adjusted to 2.50.

In any case, the loss of the important but heat-labile amino acid, lysine must be considered. This would be particularly true if reducing sugars, such as dextrose, were present in the sauce, as in many canned bean products or in home preparation. The reducing

sugar-amino acid condensation reaction may reduce the availability of lysine as a nutrient if cooking at high temperature is prolonged. A reduction in PER* has been demonstrated for pea beans coated with dextrose and cooked in steam at 121°C, as shown in Table 1. The negative PER's resulted from actual weight loss in the test rats.

A similar but less drastic reduction in nutritional value was even noted when precooked beans, given the sugar-coating necessary to control bursting or "butterflying", were subsequently subjected to high drying temperature (5).

Possible nutritional improvement of pea beans by fortification with the first-limiting essential amino acid has been considered. Supplementation of a 10% protein diet with .64% DL-methionine maintained the adjusted PER close to 2.50, the lower limit of so-called high-quality protein, through normal cooking times up to 60 min. at 121°C, as shown in Table 2. The slight loss indicated at 90 and 120 min. is probably the result of cystine destruction. The low value recorded for "O" cooked is due to the presence of the aforementioned anti-nutritional factors.

TABLE 2

Maintenance of high PER in steam-cooked pea beans by fortification with .64% methionine

Cooked time 121°C. min.	Adjusted PER
0	1.18
15	2.53
30	2.43
60	2.40
90	2.36
120	2.17

CONCLUSION

If beans have an image of being the poor man's food, I say that it is both deserved and undeserved... well-deserved because beans are easy to grow and convenient to preserve in their natural dry state... and undeserved because their being commonplace should not detract from the culinary delight they can afford. I myself was brought up on a predictable Saturday night meal of pea soup or baked beans during the thirties when my family was

* PER = Protein Efficiency Ratio, a standard measure of nutritional quality.

poor like many another, but these dishes afford no less satisfaction now in more favorable circumstances. If they lack something of convenience or appeal, it is not beyond our abilities as food technologist to provide practical remedies. We should continue to accumulate the basic knowledge on which industry can build the required technology. Of course, that effort by itself would not suffice. Advertising must then be directed toward the many who need what this food resource can offer - a beneficial combination of economy, satisfaction, and exceptional nutrition.

SUMMARY

Dry beans have been in wide use for a very long time, but we are still concerned with making them more convenient to use. Improvements often have been directed toward shortening the preparation time, and incidentally saving fuel - an increasingly important consideration everywhere. More recently, the emphasis has been on achieving this economy without excessive loss of nutritive value in the bean product. Hydration of the dry bean is necessary for the hydrolysis of carbohydrate, the denaturation of protein, and the softening of texture - all characterizing the cooking effect and required for ease of both ingestion and digestion. Procedures for speeding hydration have included soaking at elevated temperature (180°F) and the use of various inorganic salts in the soak water. Beneficial effects have been demonstrated for pretreatment with heat (steam) or cold (freezing), and for variously breaking or removing the seed coat. Hydration and cook may be merged into one continuous process where (1) dry beans are filled directly into a liquid medium in the can for long retort processing, or (2) a programmed process temperature is carefully followed to combine or connect the two operations without excessive softening effect. And finally, cooking is accomplished more quickly at the high temperatures obtained under pressure. Losses of nutrients into the processing medium (water) and degradation of nutrients such as heat-labile amino acids or vitamins at high process temperatures or in the presence of reducing sugars continue to be problems. Processing procedures should be selected to minimize these losses while affording a final product form convenient and acceptable for the use intended, stable in storage, and economical for use by low-income families.

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Production of instant bean powders

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INTRODUCTION

Beans are among the best plant proteins available. They require excessive time for soaking and cooking before consumption. Instant bean powders alleviate this problem by making a bean product available to the consumer practically instantaneously.

The main requirement of an instant bean powder is that it must reconstitute rapidly to the same texture, appearance and taste as a conventionally prepared bean soup. Only powders in which cell rupture has been kept to an absolute minimum will meet this requirement. If too many cells are broken and too much free starch is liberated, the reconstituted product will be pasty and unacceptable. For this reason instant bean powder cannot be made by dry milling whole precooked dried beans.

Instant precooked bean powder is made by a process in which the beans are soaked, cooked and finally dried. The moisture content of the cooked beans is between 50 and 55 percent, wet basis.

Drum and spray drying are the most economical drying methods for the removal of excess moisture from high moisture content food slurries. Both processes have been used successfully by the authors in converting cooked beans into rapidly rehydrating bean powder.

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Author's note: The study reported in this paper was part of a cooperative research project conducted by the Western Utilization Research and Development Division (ARS), U. S. Department of Agriculture (Albany, Calif.) and Michigan State University.

MATERIAL AND METHODS

Soaking and Cooking

The bulk of the work in this project was done with the legumes, pea beans, peas and lentils. The instantizing process has been applied to a number of other bean varieties with simular results as those reported here for pea beans, peas and lentils.

Recently harvested beans were bougth in bulk at an elevator and stored at a temperature of 40F and moisture content between 13 and 15%, wet base, for varying lengths of time, depending on when they were used for making bean powder. The first processing operation was soaking. This was followed by cooking in a retort. Through the use of taste panels, the optimum times and temperature for soaking and cooking pea beans, peas and lentils were found to be 40, 30 and 30 minutes, respectively, for soaking at 210 F, and 30, 20 and 30 minutes, respectively, for retorting at 230 F. The cooked beans were applied to the drum drier as whole beans. In the case that the beans were to be dehydrated in the spray drier, the cooked beans were wet-milled in a pulper with 0.065 and 0.023 inch sieve openings; the solids content was adjusted during the pureeing operation to whatever level was desired for a particular test.

Drumdrying

Two drum driers were used: (1) a laboratory double drum drier with 6 by 7 5/8 inch drums, and (2) a pilot scale drier with 12 by 19 1/8 drums. The heated surfaces of both driers were chrome-plated. To improve the adherence characteristics of the cooked beans to the heated drum surfaces, both driers were equipped with an automatic doctor knife-lifting device.

To achieve several applications or layers of bean material to the heated drums of a double drum drier before the dried sheet is removed the doctor knife was lifted for a predetermined number of drum revolutions.

The knife-lifting device consists of a number of appropriately connected electrical, mechanical and pneumatic components. The control unit may be remote from the drier with only electrical and pneumatic connections required. Raising and lowering of the doctor blade is accomplished by two air-operated, double-acting cylinders

mounted on the drier main frames and mechanically linked to the blade-tension arm assembly. Air-pressure regulating valves located within the control unit and pressure-indicating gages at the drier provide independent control of the pressure applied to each end of the doctor blade when in the lowered position. The drier operator controls the number of product applications made to the heated drum by positioning a selector lever at the control unit. Changing the desired number of product applications may be done at any time, even while the drier is in operation.

The knife-control device operates entirely automatically, repeating the cycle of product application and removal. The automatic knife-lifting device is described in detail elsewhere.

Spraydrying

The spray drier used in this investigation was a horizontal cocurrent-type drier. The inside dimensions of the V-shaped drying chamber were: height, 11.5 ft.; lenght, 18.5 ft., and maximum width, 7.5 ft. The inlet fan delivered 3300 cu ft of air per min at the prevailing static pressure; the capacity of the exhaust fan under those conditions was 4000 cu ft of air per min.

A positive-feed pump fed the bean slurry into the high-pressure pump of the spray drier. The positive-feed pump was required in order to prevent air from entering the high-pressure pump. The positive pump did not affect the bean-slurry capacities of the drier. Spraynozzle pressures between 500 and 3,500 psi were tested. The inlet and outlet air temperatures varied between 350 and 280 F and 220 and 160 F, respectively.

The atomization of the bean slurry was accomplished with pressuretype nozzles with a grooved-core insert. The nozzles produced a hollow-cone spray pattern. All but one of the test series was performed with high-pressure spray nozzles. Flat top cores (No. 20) having two grooves of nominal width and depth of 0.020 in. and 0.031 in. were employed in the high-pressure nozzles. High-pressure nozzles with orifice diameters of 0.0200, 0.0250, 0.0292, 0.0380 and 0.0465 in. were used.

Bean Powder

A crusher reduced a drum dried sheet of bean material into powder. The spray dried product is in the powder form already.

The dried bean powder was evaluated for moisture content (moisture meter), particle size (Tyler Ro-Tap sieve shaker), bulk density (direct weight measurement), flowability (angle of repose), viscosity (Brabender amylograph), cell structure (Blue Value index), reconstitution (rate of settling out), and consumer acceptance (taste panel).

The powders were reconstituted by adding 1 part powder to 7 parts boiling water by weight. They were stirred on a thermostatically controlled hot plate at 180°F for 15 minutes with a magnetic stirrer.

RESULTS

Drumdrying

The many experimental tests with bean puree on the conventional double-drum drier have shown that this piece of equipment is not suitable for drying pureed beans. At no time was it possible to maintain a continuous product sheet with a moisture content below 10 percent. Pureed beans can be dried on a double-drum drier equipped with an automatic knife-lifting device. For instance, for pea beans a capacity of 3.57 lb of 4.5 percent moisture content powder was obtained when 32 percent solid content bean mash was applied to a drier operating at 50 psig and 17 rpm using five layers.

Cooked beans can best be drum dried in the non-pureed form. During the pureeing process water has to be added to the beans to improve the flow characteristics of the resulting slurry. This water has to be removed in the drier, thereby adding to the energy requirements. Since whole beans can be dried equally well on a properly designed drum drier as pureed beans, it is obvious that the cooked beans should not be pureed.

A large number of laboratory tests were conducted to determine the optimum steam pressure, drum clearance and rpm settings on the capacity of the drier and the characteristics of the bean powder. Figures 1 and 2 are representative of these results.

Increased steam pressures raises production rates on a drum drier because it allows the use of higher drum speeds. The highest capacity is obtained at the highest steam pressure at which no

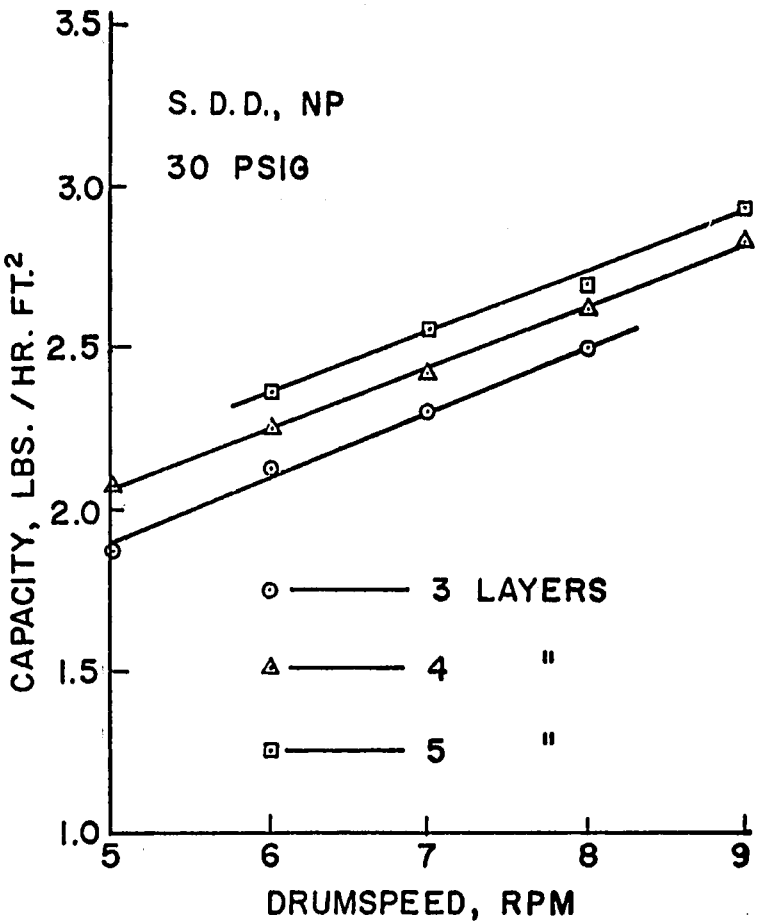

Figure 1. The effect of drum speed and number of sheet layers on the production rate of non-pureed beans on a single drum dryer, final moisture content 5.3 ± 0.5 percent.

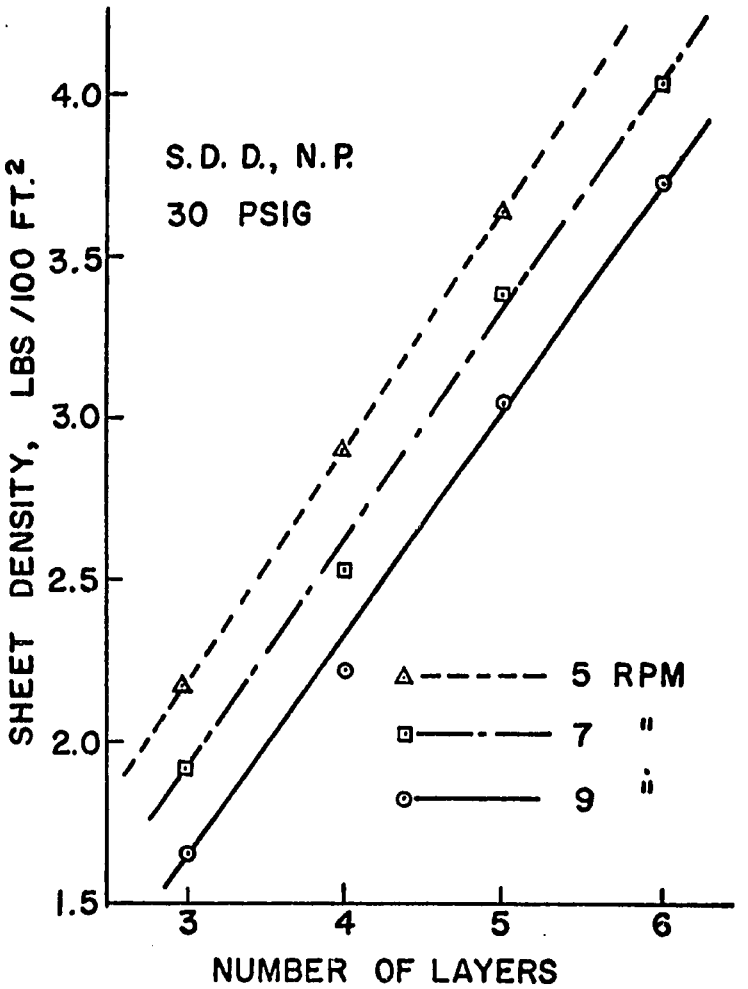

Figure 2. The effect of number of sheet layers and rpm on the sheet density of non-pureed beans on a single drum dryer, final moisture content 5.3 ± 0.5 percent.

burning of the sheet to the drum occurred. This, in general, is true for every type of bean material dried. For instance, for the non-pureed beans on the single-drum drier, the production rate of approximately 5 percent moisture content bean powder was raised from 25 to 50 to 75 psig.

The optimum condition with non-pureed material on the single-drum drier was obtained at a steam pressure of 75 psig at 23 rpm using four layers. The production rate under those conditions was 6.1 lb of solids per hour per square foot at a moisture content of 5.2 percent and a sheet density of 2.16 lb of solids per 100 sq ft.

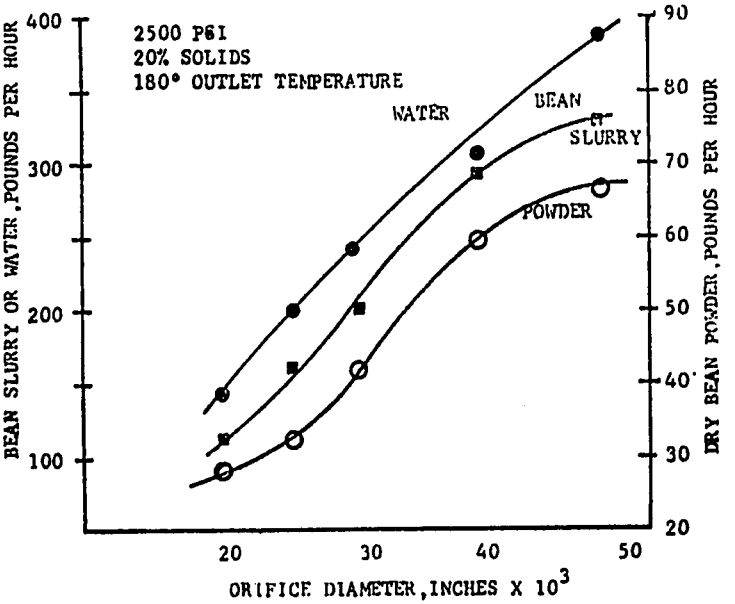

Figure 3. Spray dryer capacity of water, bean slurry and bean powder as a function of orifice diameter.

Spray Drying

Figures 3 and 4 are representative for the spray drying results. The capacity, in lbs of dry bean solids per hour and in lb of bean slurry per hour is plotted versus orifice insert diameter for high pressure nozzles. In the same figure the rated capacity in lb per hour of water is drawn. The data shows that a conversion factor of approximately 0.85 should be used in converting the nozzle capacities in gallons of water per hour to gallons of 20 percent solid content bean slurry per hour.

The effect of nozzle pressure on the capacity is illustrated in Figure 4 for a 15 percent solid content bean slurry. As in Figure 3 the rated capacity in gallons of water is included for comparison. The conversion factor for converting the nozzle capacities in gallons of water per hour to gallons of 15 percent solid content bean slurry is about 0.65.

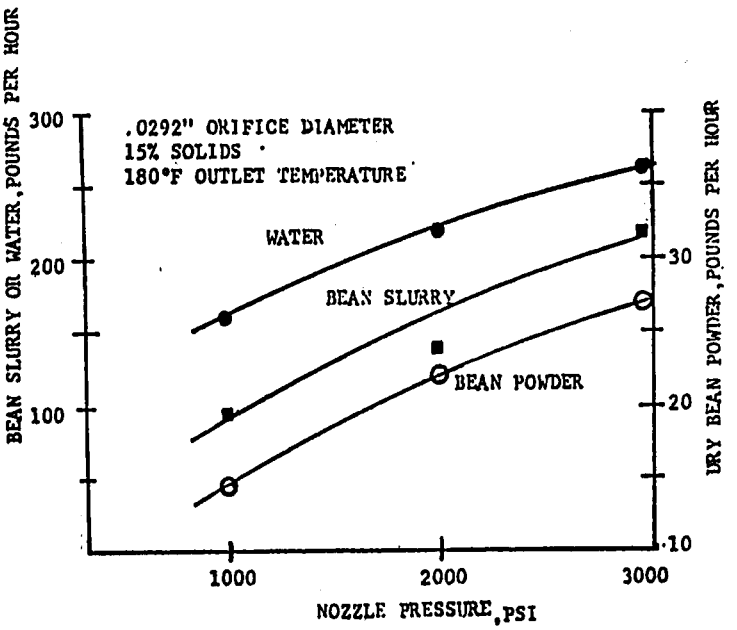

Figure 4. Capacity versus nozzle pressure.

Bean Powder

The viscosity, blue value index, and other mechanical, physical and organoleptic values for pea bean, lentil and pea powder will be presented elsewhere in detail (2). It can be reported here that both the spray dried and drum dried legume powders were pudged highly acceptable by consumers panels. The particle size of the spray dried powders and therefore also bulk density and other properties, are dependent upon the drying conditions. The bulk density, reconstitution, and flowability of the drum dried powders could be controlled by the screen size of the mill used to pulverize the dried sheet from the drum drier. Microscopic examination and blue value index values indicated that very little cell rupture and liberation of free starch had occurred in both spray dried and drum dried powders.

Off-flavors did not develop in powders stored at room temperatures for 12 months at 4 to 5 percent moisture in the presence of air but did occur at moisture contents above 6 percent.

Economics

Although acceptable instant bean powders can be produced economically with both a drum drying and spray drying system, the drum drier is to be preferred as the dehydrating device mainly for economic reasons. Therefore, the cost of the drum drying system will be presented for the manufacturing of pea bean, pea and lentil powder.

In determining the manufacturing cost of drum dried pea bean, pea and lentil powder, it has been assumed that the plant is operated on a twelve months a year basis - nine months on legumes, three months on some other products, such as potatoes, sweet potatoes, or mushrooms.

The plant will produce each of the three legume powders durings 83 days (three months) a year, 6 days a week and 23 hours a day. The hourly, daily and three-monthly capacities of the plant for the three legumes in terms of raw product and dry powder are

pea bean powder	5.5 lbs per ft^2 per hour
pea powder	4.0 lbs per ft^2 per hour
lentil powder	2.5 lbs per ft^2 per hour

The legumes will be bought in one hundred pound bags and will have been cleaned when they arrive at the plant. The assumed f.o.b. cost of the beans, peas and lentils is $ 9.50, 6.35, and 11.50 per hundred weight, respectively. Although split and broken legumes are sometimes available for as little as $ 3.25 per hundred weight and can be used successfully, this appreciable savings in cost has not been included in the cost analysis due to the uncertain supply source.

It has been assumed that the plant will be completely new. If the dehydration plant can be added to an existing processing plant, both the initial capital costs and the operating costs would be lower because certain facilities could be shared. Thus, the costs shown for producing bean, lentil and pea powder are high if a partial use is made of already existing facilities.

The legume powders will be sold for remanufacturing or institutional use and be packed in 20 in. diameter by 25 in. high

fiber drums. The drums will have lever closed, gasketed lids with a built-in 4-mil polyethelene liner as moisture barrier and will hold 125 pounds of powder with a density of 30 pounds per cubic foot.

The individual pieces of equipment required in a legume powder manufacturing plant are shown in Figure 5. The total equipment and capital cost required for producing pea bean, pea and lentil powder are the same for the three products: $ 147, 500 and $ 749, 500 respectively. The capital cost are based on plant cost estimate information and economic indicators (cost index figures) regularly published in Chemical Engineering. The capital costs include items ranging from land and site preparation to buildings, steam generation equipment, offices, engineering fees and working capital.

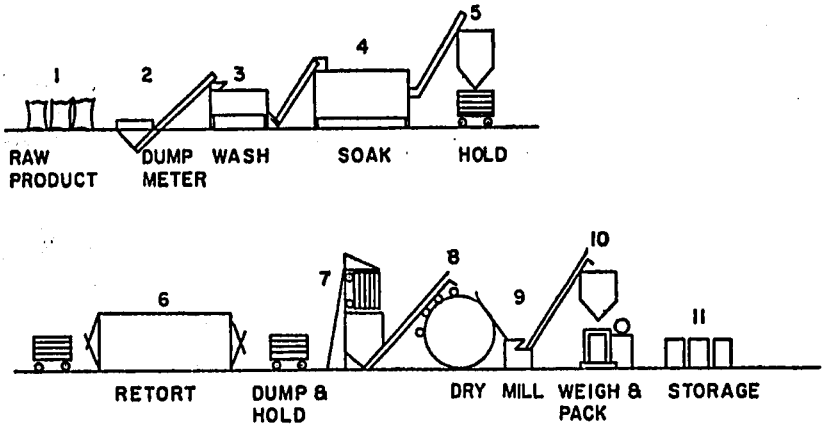

Figure 5. Flow sheet of legume powder manufacturing process

The operating cost for manufacturing legume powder include the direct production costs (raw material, packaging, labor, utilities, etc.), indirect cost (insurance, taxes, interest on fixed capital, depreciation, etc.), plant overhead (payroll overhead, waste disposal, etc.) and general expenses (interest on working capital, administration).

The operating costs for manufacturing instant pea bean, pea and lentil powder is given in Table 2. These prices are up substantially (due to inflation and increase in labor costs) since a similar study was made on the economics of instant legume powders (3).

At daily production rates of 31,800 lbs of bean powder, 23,200 lbs of pea powder and 14,500 lbs of lentil powder the total capital required is about $ 900,000. The total cost to make one pound of pea bean powder is approximately 20 cents, of pea powder 19 cents, and of lentil powder 33 cents under 1973 U.S. conditions (4).

TABLE 1
PROJECTED HOURLY, DAILY AND TRI-MONTHLY CAPACITIES OF 4.5 PERCENT MOISTURE PEA BEAN, PEA AND LENTIL POWDER; INITIAL RAW PRODUCT MOISTURE CONTENT 14 PERCENT

	Raw Product, lbs x 1000			Powder, lb x 1000		
	hour	day	season	hour	day	season
pea beans	1.38	31.8	2,640	1.24	28.6	2,370
peas	1.01	23.2	1,930	0.91	20.8	1,730
lentils	0.63	14.5	1,200	0.27	13.7	1,080

TABLE 2
OPERATING COSTS FOR LEGUME POWDERS, CENTS PER POUND

	pea beans	peas	lentils
direct production costs	17.67	15.48	26.99
indirect costs	1.04	1.42	2.34
plant overhead	.58	.80	2.19
general expenses	.73	1.01	1.59
cost to make	20.02	18.71	33.11

SUMMARY

Two processes for manufacturing instant legume powder with good flavor and reconstitution characteristics have been developed. The process was tested with a number of bean species; engineering, physical, economic and organoleptic investigations were limited to pea beans, peas and lentils. The main operations in the instant bean powder process are soaking, retorting and dehydration. Properly stored pea beans are soaked, retorted and dehydrated in a spray or drum drier. An automatic doctor knife-lifting device insured satisfactory adherence of bean material to the steam-heated drums of the drum drier. The legume powders were pudged by consumer panels as highly acceptable. The bulk density, reconstitution, and flowability of the powders could be controlled by the screen size of the mill used to pulverize the dried sheet from the drum drier. Microscopic examination and blue value index values indicated that very little cell rupture and liberation of free starch had occurred in the powders. Off-flavors did not develop in powders stored at room temperatures for 12 months at 4 to 5 percent moisture in the presence of air but did occur at moisture contents above 6 percent. An economic study based on laboratory and pilot scale data showed that pea bean powder can be produced for about 20 US dollar cents per pound under 1973 USA conditions.

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Effect of cooking and germination on the flatus inducing capacity of some legumes

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INTRODUCTION

Legumes form part of the daily diet of people in India and consumption of larger quantities of legumes is recommended for increasing the protein content of cereal based vegetarian diets. Legumes have also been used in the preparation of low cost protein foods (1) weaning foods (2) and protein foods used in the treatment of kwashiorkor (3). However ingestion of large quantities of legumes is known to cause flatulence in experimental animals and humans (4-7). This property of legumes may hinder their wider use. Our earlier observations have shown that inclusion of extra amounts of legumes in the diet of children induced increased amounts of flatus (8). Since it may not be possible to carry out all the studies on humans, the present studies were undertaken to see whether the response is similar in rats. In addition, since some of the legumes are consumed in raw, cooked and germinated form, the effect of cooking and germination on the flatus inducing capacity of some commonly consumed Indian legumes was studied.

MATERIALS AND METHODS

Whole legumes and *dhals* (dehusked, split legume) of *Bengal gram (Cicer arietinum), green gram (Phaseolus radiatus), Black gram (Phaseolus mungo)* and *Red gram (Cajanus cajan)* obtained from the local market were used.

Dhals Bengal gram and *green gram* are cosumed in raw (soaked till soft) and cooked forms, hence dhals were soaked or cooked till soft, dried at 50°C and powdered.

Whole legumes of *Bengal gram* and *green gram* were germinated for 48 hours, dried at 50°C and powdered.

In the first series of experiments, 5 groups of albino rats, 10 in each group were fed a synthetic diet containing 18 per cent casein and 69 per cent corn starch or containing 65 per cent of dhals of the four legumes mentioned above. The animals were fed the diets for 10 days and the volume of intestinal flatus was measured according to the method of Hedin (9).

In the second and third series of experiments, rats were fed casein diet or diets containing 65 percent of raw or cooked or germinated *green gram* and *Bengal gram* and the intestinal flatus volume measured as described earlier.

RESULTS

The results obtained on the intestinal flatus volume of the rats are given in Tables 1 and 2.

TABLE 1

Intestinal flatus of rats * fed different legume based diets.

Diet	Flatus volume (ml)
Casein	0.59 ± 0.16
Bengal gram dhal	1.99 ± 0.22
Green gram dhal	1.28 ± 0.21
Black gram dhal	1.61 ± 0.19
Red gram dhal	2.04 ± 0.25

* Weighing 150-180 g and consumed on an average 5 g of diet.

TABLE 2

Intestinal flatus of rats * fed diets containing raw, cooked and germinated legumes

Diet	2nd series **		3rd series	
	Raw	Cooked	Raw	Germinated
Casein	1.13 ± 0.20	—	—	—
Bengal gram	2.30 ± 0.23	2.24 ± 0.19	2.36 ± 0.23	1.90 ± 0.23
Green gram	1.91 ± 0.25	1.79 ± 0.26	2.08 ± 0.18	1.62 ± 0.20

* Weighing 200-250 g and consumed on an average 8 g of diet.
** Dhals were used

The results clearly indicate that the diets containing *dhals* of *Bengal gram, red gram, black gram* and *green gram* produced large amounts of intestinal flatus as compared to a basal casein diet. Among the *dhals* the volume of flatus produced was in the following decreasing order. *Red gram* > *Bengal gram* > *Black gram* > *Green gram.*

Between the raw and cooked *dhals* of *Bengal gram* and *green gram* not much difference is seen in the volume of flatus produced.

Germinated legumes show a very slight reduction in the flatus volume as compared to raw legumes.

DISCUSSION

The observation that inclusion of legumes in the diet increases the flatus production is in conformity with the results of earlier workers (4-7). The differences seen in the flatus producing capacity of the different legumes is almost similar to the results we have reported earlier with humans (8). The flatulence has been ascribed to the presence of stachyose and raffinose in the legumes but the legumes under the present study contain similar amounts of these sugars (10) and yet *green gram* produced smaller amounts of flatus as compared to *Red gram* and *Bengal gram*. Calloway *et. al.* (11) also found that white beans, containing lesser amounts of oligosaccharides than soya beans produced more flatus and enzymatic treatment of soya beans designed to hydrolyse raffinose and stachyose also had only negligible effect on the production of intestinal gas. At the same time when the total carbohydrate contribution was equalized, both soya bean and white beans produced similar amounts of flatus. *'Tofu'*, which contains most of the initial protein and fat as in the original soya bean but little carbohydrate was found to contain very little flatulence factor. This points out to the fact that besides oligosaccharides, total carbohydrates may be involved in the process of flatus production.

In our studies with rats (unpublished data) we observed that carbohydrates of legumes under study were less digestible as compared to corn starch and *green gram* was the most digestible and *Bengal gram* and *Red gram* the least. Rao (12) also observed the same. The cecum of the rats fed these legume diets were found to be enlarged and faecal bulk also was increased and this could be due to the indigestible residues (13). The flatus inducing capacity of the legumes somehow appears to be correlated to the digestibility of the legumes. *Green gram* which is the most digestible produced the least amount of flatus and *Bengal gram* and *Red gram* the least digestible produced the maximum volume of flatus.

Cooked *green gram* and *Bengal gram dhals* retained most of their flatus inducing capacity. This observation is in conformity with that of Hellendoorn (6), where cooking of beans for varying lengths of time did not affect the flatus inducing property of beans.

Rao (12) observed that *in vitro* digestibility of the carbohydrates of *Bengal gram* and *Green gram* improved on cooking, but the difference in the digestibility of these legumes was maintained. This may explain the retention of most of the flatus inducing property in the cooked legumes.

Germination of *Bengal gram* and *green gram* did not much alter the flatus inducing capacity. The results are in conformity with those of Murphy & Calloway *et al* (11) where sprouting of white beans, mung beans and soyabenas did not affect their flatus inducing property. During 48 h of germination of soya bean the amount of stachyose and raffinose reduces by 50 per cent (14) and completely disappears in *green gram* (10) and yet germination of legumes does not alter the flatus inducing capacity. This also points to the fact that there may be factors other than oligosaccharides that are involved in the flatus production.

ACKNOWLEDGEMENT

The authors gratefully acknowledge Dr. M. Narayana Rao, who initiated this present work and Dr. M. R. Raghavendra Rao for guidance.

SUMMARY

Feeding of diets containing four dhals (dehusked, split legume) of Bengal gram (Cicer arietinum), green gram (Phaseolus radiatus), Black gram (Phaseolus mungo) and Red gram (Cajanus cajan) to rats produced more intestinal flatus as compared to a casein diet. Among the legumes green gram was found to produce the least amount of flatus. Cooking of dhals of Bengal gram and green gram and germination of the two legumes did not greatly alter their flatus inducing capacity, as compared to the raw forms.

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Effect of germination and maturation on the nutritive value of common beans (Phaseolus vulgaris)

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INTRODUCTION

Very few studies have been carried out on the nutritive value of legume seeds during the different physiological stages of the seeds in maturation and germination. As far as the germination process is concerned, contradictory results have been reported. Some investigators have found an increase in the nutritive value during germination (1, 2), others indicate no change or a decrease (3, 4), while still in other studies the results are not conclusive (5).

Most of the research on the nutritive value of legume foods has been carried out on the mature seeds (6) probably because this is the most common stage of consumption. In general the results have indicated that legume seeds are relatively of poor nutritive value, which has been attributed to the presence of heat-labile, antinutritional factors —which includes trypsin inhibitors, hemaggutinins, and other (7, 8)— to their deficiency in the sulphur containing amino acids (9, 10), and to their low protein digestibility (6). Despite these findings, several investigators (6) have found differences in the nutritive value between species as well as between varieties or cultivars of the same species, which have not been elucidated. Therefore, it is expected that a better understanding of the physiological and biochemical processes that

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occur at different stages of seed development could be helpful in explaining the results obtained with dry beans, and it may contribute to improve their protein utilization through appropriate genetic selection. The work described here was undertaken to determine the effect of germination and of maturation on the gross chemical composition and nutritive value of black beans *(Phaseolus vulgaris)*.

MATERIALS AND METHODS

Samples of common beans *(Phaseolus vulgaris)* of the variety S-19N were studied at different stages of germination and maturation of the seed. The design of the study is shown in Figure 1.

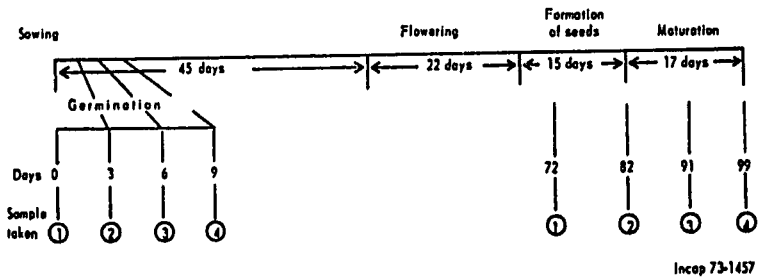

Figure 1. Vegetative period of black beans (Phaseolus vulgaris)

Treatment of the samples

1. *Germination*

For the germination study samples were taken at 0, 3, 6 and 9 days. For each treatment 7.8 kg of sterilized seeds were germinated in a dark laboratory room, using individual metal trays of 98 x 118 cm for each of three replicates. The average recorded temperature in the room was 25°C. Each sample was immediately refrigerated then freeze dried and stored in a cold room until needed for the chemical analyses and biological assays. Part of each sample was cooked in an autoclave during 10 minutes at 16 lb pressure (121°C) and dried in an air oven for 22 hours at 50°C. The raw dried, and cooked dried samples were ground in a Wiley mill to pass 40 mesh.

2. *Maturation*

This part of the experiment was carried out at the INCAP experimental farm. During maturation, 4 samples were studied at 8 - 10 day intervals. The first sample was taken 72 days after

sowing or 5 days after flowering, and then at 82, 91 and 99 days of the second, third and fourth samples, respectively. Each sample was brought to the laboratory immediately after harvest, and the seed was separated from the pods. The seeds were treated in a similar way as described previously for the germination study.

3. *Chemical analyses*

The moisture, nitrogen, ether extract, crude fiber, ash and calcium were determined by the A.O.A.C. methods (11).

Phosphorus content was measured by the technique of Fiske and Subbarow (12) modified by Lowry and Lopez (13), and iron, according to the procedure of Jackson (14), and Moss and Mellon (15). Nitrogen solubility was determined according to the methodology described by Hasbun (16); lysine was estimated by the method of Conckerton and Frampton (17), while methionine and cystine were assayed by using microbiological procedures with Difco media (Difco Laboratories, Detroit, Michingan), and *Leuconostoc mesenteroides* P-60. Free amino acids were estimated as α-NH_2-Nitrogen (18).

4. *Experiments with rats*

Diets. Two of the experiments were carried out to determine the effect of growth inhibitors and/or antiphysiological factors on the growth of rats. In this case the raw samples were used. In other biological trials, the protein efficiency ratio (PER), and digestibility in the cooked samples were determined. In both cases the dry beans samples were added to provide about 10% of protein in the diets, which consisted of (%): mineral mixture (19), 4.0; cod liver oil, 1.0; soybean oil, 5.0 and sufficient corn starch to adjust to 100. All diets were supplemented with 5 ml of a vitamin solution (20).

Rats. The rats used were of the Wistar strain from the INCAP colony.

Protein Efficiency Ratio. For the germinated samples 6 male rats per group were used, while for the samples collected at various stages of maturation, 8 animals per group were used, of which four were male and 4 female. The animals within each sex were randomly distributed by weight among the several experimental groups, and the mean weight among the groups differed by not more than 1 g. The animals were kept in individual all-wire cages with raised screen bottoms. Food and water were given *ad libitum.* The complete experiment lasted 28 days, except in the case of the raw samples where the experimental period depended on the

survival time of the rats; gains in weight and food intake were recorded weekly. At the end of the experimental period the PER (g gained/g protein intake) were calculated.

Growth inhibitors. The concentration of growth inhibitors was assessed by calculating the percentage of survival time of the animals fed the different raw bean samples during the 28 day experimental period.

Digestibility. Protein digestibility was determined at the end of the PER experiments. For this purpose the animals were kept in the same experimental cages and were offered the same diets under test; the balance period was of 7 days on each experimental diet. Feces were collected daily and stored under refrigeration. Nitrogen was determined by the Kjeldhal method (11). The formula used to calculate the apparent digestibility was (nitrogen intake — fecal nitrogen)/nitrogen intake x 100 = apparent digestibility (%).

RESULTS

The average chemical composition of beans at different physiological stages on a fresh weight basis is shown in Table Nº 1. As can be seen, during germination there is an increase in the water content, and a decrease in the amount of protein, fat, crude fiber and ash. Protein content decreased from 20.0 to 4.8 g%, and water increased from 16.6 to 82.1 g%. On the other hand, during maturation, water content decreases progressively and the other nutrients increase.

TABLE 1
CHEMICAL COMPOSITION OF COMMON BEANS (Phaseolus vulgaris) AT DIFFERENT PHYSIOLOGICAL STAGES
(g%)

Days of germination	H_2O	Protein	Fat	Crude Fiber	Ash
0	16.6	20.0	1.04	5.3	3.8
3	56.8	10.5	0.92	2.0	1.8
6	69.4	7.6	0.68	1.8	1.2
9	82.1	4.8	0.41	1.6	0.7
Maturation sample no.					
1 (72 days)	74.9	7.2	0.57	1.6	1.6
2 (82 days)	56.4	11.9	0.92	2.6	2.3
3 (91 days)	54.9	12.8	1.02	2.7	2.2
4 (99 days)	15.2	23.1	1.11	4.4	4.7

TABLE 2
MINERAL COMPOSITION OF COMMON BEANS (Phaseolus vulgaris) AT DIFFERENT PHYSIOLOGICAL STAGES*

Days of germination	Ca mg %	P mg %	Fe mg %
0	200	477	5.4
3	92	214	2.4
6	74	148	1.8
9	47	106	1.2
Maturation Sample no.			
1 (72 days)	74	125	1.4
2 (82 days)	50	265	2.4
3 (91 days)	122	305	2.4
4 (99 days)	186	504	5.9

* All values are expressed on fresh basis.

TABLE 3
LYSINE, TOTAL SULPHUR AMINO ACIDS AND FREE AMINO ACID CONTENT OF COMMON BEANS (Phaseolus vulgaris) DURING GERMINATION AND MATURATION

Days of germination	Lysine g/16 g N	Total sulphur amino acids* g/16 g N	Free amino acids** mg %
0	6.12	1.44	25
3	5.77	1.25	154
6	5.72	1.21	262
9	4.87	1.15	279
Maturation sample no.	**Lysine g/16 g N**	**Total sulphur amino acids g/16 g N**	**Free amino acids mg %**
1 (72 days)	5.33	1.27	80
2 (82 days)	5.52	1.51	56
3 (91 days)	5.95	1.61	42
4 (99 days)	7.10	1.40	32

* The lower in total S.A.A. was mainly due to a decrease in cystine content.

** Measured as α NH_2 - Nitrogen.

Table Nº 2 shows the mineral composition of the samples both, during germination and maturation. As with the other nutrients, the results indicate that sprouting decreases the amount of calcium, phosphorus and iron, while during maturation the reverse is observed.

Lysine, total sulphur amino acid and total free amino acid content is shown in Table Nº 3. Free amino acids measured as α NH_2 — Nitrogen increased in the germinative stages, while they decrease during maturation. Lysine and total sulphur amino acids decrease to about the same relative extent during germination. On the other hand, an increment in the content of these same amino acids was observed as the beans mature.

Figure 2 shows the results of a continuous protein extraction of beans with different solvents at several stages of development. Solubility in 0.01 N NaOH decreased with germination while an increase was observed during maturation, whereas the fraction extracted with 5% KC1 increased with germination and decreased with maturation. Protein solubility in 70% ethyl alcohol and water, remained practically unchanged. The data also indicate that protein extraction was higher with 0.01 N NaOH solution, followed by the 5% KC1, water and alcohol.

The effect of growth inhibitors on the survival time of rats fed the raw samples is illustrated in Figure 3. Percentage survival time was calculated by expressing the actual survival time of each group, with respect to 28 days experimental period, taken as 100% survival time.

As it can be seen, the percentage survival time increased during germination from 30% to about 60%, indicating a lower content of toxic factors as germination progressed. The opposite was observed during maturation. In this case survival time changed from about 30% to 20%.

Table Nº 4 shows the changes in the nutritive value of common beans at different stages of sprouting. The results indicate a significant decrease in the protein quality during germination, as can be observed from the lower weight gains and PER values. The apparent digestibility was not significantly affected, although there was a tendency to decrease.

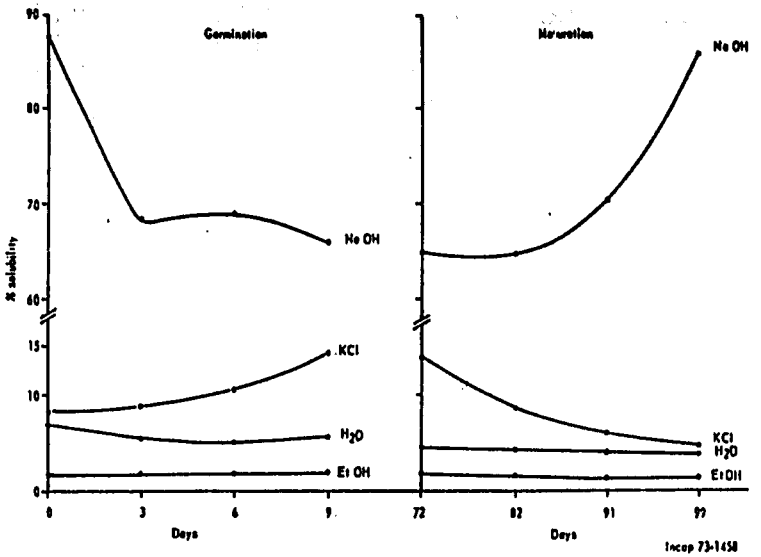

Figure 2. Protein solubility of common beans (Phaseolus vulgaris)

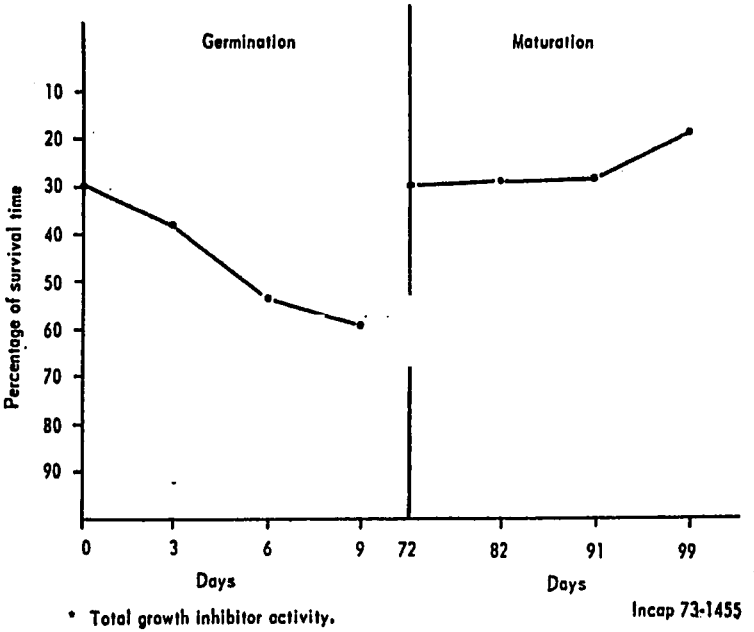

Figure 3. Relationship between trypsin inhibitors and survival time of rats fed raw common beans (Phaseolus vulgaris) at different physiological stages.

TABLE 4

CHANGES IN THE NUTRITIVE VALUE OF COMMON BEANS (Phaseolus vulgaris) DURING GERMINATION*

Days of germination	Protein in diet %	Average initial g	Weight gain in 4 weeks g	P.E.R.**	Apparent Digestibility %
0	10.4	53	28	0.99	67
3	9.9	53	24	0.86	64
6	10.3	53	15	0.59	67
9	10.2	53	4	0.26	60

* All samples were cooked in the autoclave during 10 minutes, at 16 lbs pressure. (121°C).

** P.E.R. = Protein Efficiency Ratio. Mean values for six rats/group.

Table Nº 5 presents the results on the nutritive value of common beans during maturation. From both weight gain and PER values, it is evident that there is a tendency for nutritive value to decrease with the progress of maturation. On the other hand, digestibility shows a tendency to increase. It should be indicated at this moment, that the 99 day mature sample corresponds to the 0 day germination sample in theory. Therefore, protein quality should be practically the same. Our results show different values, in both weight gain and PER. We do not have a complete explanation for this difference. However, the samples that were used for the germination studies, which at 0 time gave higher weight gain and PER, were sun dried after harvest. Experimental evidence showed that this common practice carried out by farmers, improves the protein quality of beans.

TABLE 5

CHANGES IN THE NUTRITIVE VALUE OF COMMON BEANS (Phaseolus vulgaris) DURING MATURATION*

Maturation sample no.	% Protein in diet	Average Weight initial g	gain in 4 weeks g	P.E.R.	Apparent Digestibility, %
1 (72 days)	9.9	46	30	0.97	61
2 (82 days)	9.8	46	19	0.75	68
3 (91 days)	9.6	46	15	0.67	64
4 (99 days)	9.6	46	16	0.63	68

* All samples were cooked in the autoclave during 10 minutes at 16 lbs pressure, (121°C).

** P.E.R. = Protein Efficiency Ratio. Mean values for eight rats/group.

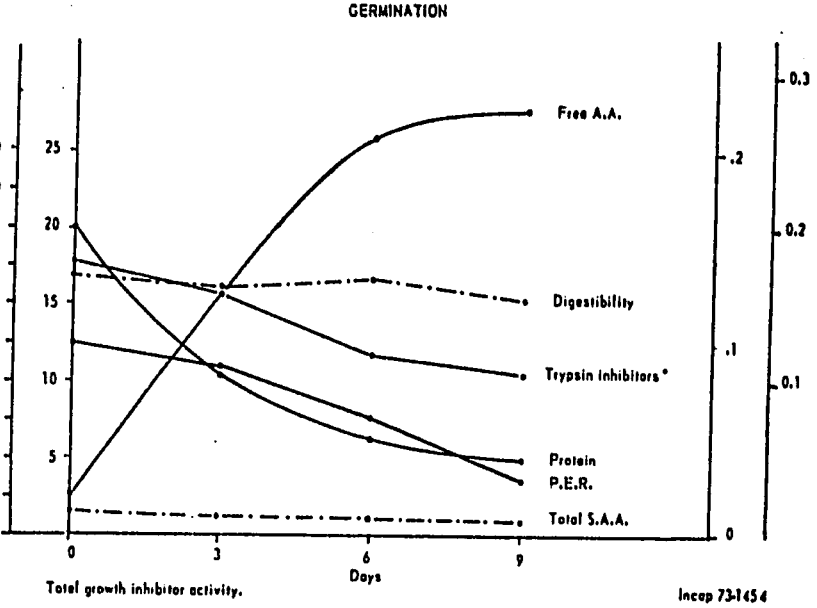

Figure 4. Germination

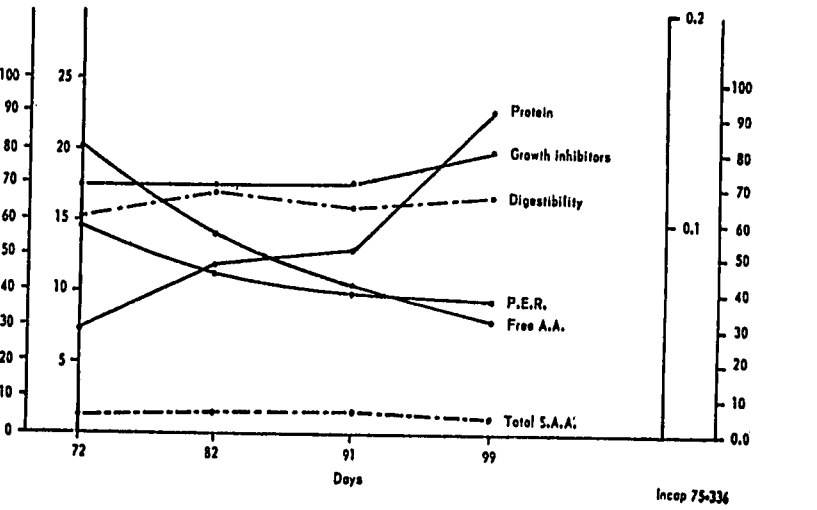

Figure 5. Maturation

Figure 4 summarizes the biochemical and nutritional changes observed during germination. As it is shown, free amino acids increase, while total protein content decreased progressively. Growth inhibitor activity also decreased as germination progressed, being more marked between the third and sixth day.

The lower nutritive value is correlated with a decrease in the total sulphur amino acid content whereas protein digestibility remained fairly constant. Figure 5 shows the results obtained during maturation in relation to the same parameters. There is a progressive increase in the total protein content, which is more marked between the 91st and 99th days, whereas free amino acid content decreased. These changes correlate with an increment in the growth inhibitor activity and the total sulphur amino acids. As in the case of germination, protein digestibility was not significantly altered, while the nutritive value decreased progressively.

DISCUSSION

As is shown in Figure 1, the vegetative period of common beans *(Phaseolus vulgaris)* can be broadly divided into four stages of development: germination, flowering, formation of the seeds and maturation. The results obtained on the chemical composition of beans reported on fresh weight basis, during the different stages of germination reflect the metabolic activities of the seed in order to provide the nutrients needed to the formation of the different vegetative parts of the new plant. In general, these results are in agreement with the studies of Pusztai (21); this investigator indicates that the first 4 days are characterized by a marked protein degradation phase, followed by a pronounced stage of new protein synthesis, reaching its maximum at about the 8th day after the start of germination. On the other hand, to start the formation of new seeds, these nutrients have to be mobilized from the plant, to the seeds; furthermore an increase in the concentration of these nutrients is to be expected with the progress of maturation. Of interest are the results obtained in relation to the presence of growth inhibitors, and the nutritive value. As far as the germination stages are concerned, the data obtained on the total growth inhibitor activity confirm the findings of other investigators. Ambe and Sohonie (22) reported the presence of trypsin inhibitors in all parts of the germinating seeds at all stages of growth. They also found a decrease in the cotyledons and an increase in the sprouts with the progress of germination.

More recently, Palmer *et al* (23) reported that both the trypsin inhibitors and the nutritive value increased during germination. This apparent discrepancy with out results may be due to the different methodology employed. Our findings indicate a decrease in the activity of growth inhibitors as germination progressed, measured by the survival time of rats, which could be due to factors other than trypsin inhibitors; these results are in agreement with the finding of Palmer *et al* (23). It is possible that at this physiological stage, the seeds possess a powerful enzymatic system that can degrade the growth inhibitors; this is suggested by the increment in the free amino acids, and the decrease in the total sulphur amino acids, content that has been reported to be higher in some toxic constituents of beans (8).

Further studies, on this enzymatic system may have in the future some practical importance.

The results on the protein fractionation can offer an explanation for the decrease in lysine content during germination since protein solubility in NaOH decreases and this fraction is known to be a rich source of this amino acid.

Studies on the fractioning of the various proteins from legumes at different physiological stages can be useful to explain the low digestibility of this group of foods.

Nutritive value was determined in the cooked material, thus eliminating the presence of the most toxic heat-labile factors present in beans. It is possible that the data obtained by Palmer *et al* (23) was influenced by the type of material employed, since they used the raw germinated seeds and casein as the source of protein in the diets.

The decrease in nutritive value during germination, can be explained by a decrease in the total sulphur amino acid content, since they are the most limiting amino acids in bean protein (9, 10, 24). From these results, it also seems that the low digestibility in cooked beans is not only associated with heat-labile toxic factors, since the former remained fairly constant during germination. It is also of interest to note that although digestibility was not affected, the nutritive value decreases significantly; previous results reported by Bressani *et al* (9) indicate that the nutritive value of black beans *(Phaseolus vulgaris)* is improved by methionine supplementation whereas digestibility remained unchanged.

The development of different pigments during maturation may offer an explanation to the increased activity of toxic constituents

at this physiological stage, since previous work has demonstrated (25) the effect of pigmentation on the toxicity and nutritive value of different cultivars of beans. More difficult, however, is to explain the decrease in the nutritive value, since the results show an increase in total sulphur amino acid content as maturation progresses.

It would seem that further studies on the sequence of other amino acids that are limiting the utilization of bean protein, are needed.

The decrease in nutritive value can also be due to the presence at these physiological stages of toxic constituents more resistant to heat. Jaffé and Hanning (26) and Seidl *et al* (27) isolated a globulin fraction from black beans *(Phaseolus vulgaris)* which was only slightly affected by heat.

As in the case of germination, the changes in digestibility during maturation were not significantly altered, indicating again the effect of other factors on the nutritive value of beans. The role of toxic factors, as well as more detailed information on the effect of the different protein fractions, and pigments on the digestibility and the nutritive value of beans, will be the subject of future studies.

SUMMARY

The effect of germination and maturation on the nutritive value of common beans (Phaseolus vulgaris) was studied. The germinated bean samples were taken at 0, 3, 6 and 9 days of germination. During maturation 4 samples were studied at 10 day intervals. During germination there was an increase in water content and a decrease in the amount of protein, fat, crude fiber and ash on a fresh basis. The decrease in protein was correlated with an increase in the free amino acid, lysine and total sulphur amino acids content while the trypsin inhibitors decreased during germination. Protein solubility in NaOH decreased whereas protein soluble in KCl increased. Water and alcohol soluble fractions remained essentially the same.

Nutritive value decreased progressively as beans germinated. Maturation increased protein, fat, crude fiber and ash and decreased water and free amino acid content. Lysine, total sulphur amino acids and trypsin inhibitors increased during maturation. Protein solubility in NaOH increased. Solubility in KCl decreased whereas water and alcohol soluble fractions remained practically unchanged. Nutritive value decreased with maturation. Protein digestibility was not affected either by germination or maturation. The results suggest that during germination the decrease in nutritive value is possibly due to the lower amounts of total sulphur amino acids. It was also concluded that at this physiological step the seeds posseses a powerful enzymatic system that can hydrolize the trypsin inhibitors, which may have practical importance. In the case of maturation the lower

nutritive value is explained by the presence of toxic factors which are heat stable and are present in the different pigments formed during maturation. It was also concluded that digestibility was not affected by the parameters studied. Finally, the results show the importance of further biochemical and physiological studies in order to assess the role of the toxic factors, protein fractions and their amino acid composition on the digestibility and the nutritive value of legume foods.

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Protein-starch extraction and nutritive value of the jack bean and jack bean protein isolate

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INTRODUCTION

Although approximately 13,000 species of legume seeds have been identified, only about 20 are of some economical importance as a food item in Latin America. In Central America and Mexico the number is even smaller.

Among the legume seeds which are either not consumed or consumed at relatively low levels, and ony in specific regions, is the Jack bean *(Canavaia ensiformis)*. This legume can be grown relatively easily, producing high yields in regions of low altitude, that are inadequate for the growth of other common legume seeds such as *Phaseolus vulgaris* (1). Thus, the Jack bean has a high potential in regions of varying climates and altitudes, as is the case for most of the Latin America areas, where it would not compete with the common bean *(Phaseolus vulgaris)* and could still provide an additional protein source.

The lack of use of the jack bean as a human food can be attributed partly to the presence of growth inhibiting factors (2, 3). The main reason, however, lies in the regional dietetic habits, that do not include this legume as part of the common diet.

Production of a protein concentrate from jack bean, which could be used in several food formulations, has been considered as a possibility to utilize this legume as a protein source for human consumption in the area (4).

However, a relatively high starch content has been reported for other legume seeds (5, 6), and a simple process has been successfully designed for the combined protein and starch isolation from

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the Mungo bean *(P. mungo)* (7,8). Thus, we considered it worthwhile to investigate the possibility of designing a similar process to isolate the two major components of the Jack bean —protein and starch— and by this means, offer a possibility for its industrial utilization.

The present work was therefore undertaken to establish the optimum conditions for extraction and recovery of the protein and starch from the Jack bean, through a simple low-cost process. A second purpose was to determine some of the nutritional characteristics of the protein and of the protein isolate obtained thereof.

MATERIALS AND METHODS

Samples and analytical procedures

The Jack bean used in this study was grown at INCAP's experimental farm "San Antonio Pachalí", Guatemala, at an altitude of 4,500 ft above sea level, and it corresponded to the 1972 crop.

Nitrogen, ash, ether extract, moisture, crude fiber, total solids and starch were determined in duplicate according to the AOAC (9). Protein was calculated by multiplying the nitrogen content by the customary conversion factor of 6.25. Total soluble sugars were determined in the alcohol washings of the starch analyses, by the phenol-sulfuric method acording to Dubois *et al.* (10), using glucose as standard.

Isoleucine, leucine, lysine, phenylalanine, threonine, tyrosine, tryptophan and valine were determined in an amino acid auto analyzer following the methods of Spackman, Stein and Moore (11). Methionine and cystine were determined microbiologically following the methods described by Elias, Colindres and Bressani (12).

Biological trials

The protein efficiency ratio (PER) of both raw and cooked (15 psig, at 121°C for 20 minutes) Jack bean seed was evaluated according to the AOAC (9). Weanling rats of the Wistar strain, from INCAP's animal colony, were distributed in groups consisting of 3 males and 3 females, each. All diets were supplemented with 4% salt mixture (13), 5% cottonseed oil, 1% cod liver oil and corn-starch to 100 g, to which 5 ml of a vitamin solution (14) were added.

Protein-starch extractions

The Jack bean used in the extraction trials was peeled either by hand or through air separation or flotation of the peel after coarse milling of the seed. The meat thus obtained was milled in a Wiley mill, equipped with a 30-mesh screen, to obtain the meal which was used throughout the extraction experiments.

All extractions were carried out in triplicate using a reciprocal water-bath shaker (New Brunswick Scientific, Model R-76) with a constant agitation equivalent to 120 strokes per minute. The time used for each extraction was 1 hour, since Molina, Argueta and Bressani (4) have established this as the minimum time to achieve the maximum protein extraction from Jack bean. The solvent: meal ratio was either 100:3 or 100:6.

The recuperation of the protein extracts (supernatant) was carried out by centrifugation at 4,500 rpm 20 minutes. The starch remained as the residue from the centrifugation process. The protein isolate was obtained through isoelectric point precipitation at the pH determined as the isoelectric point for the Jack bean proteins by Molina, Argueta and Bressani (4). The protein isolate obtained by this means was washed 3 times with water, adjusted to a pH equivalent to the isoelectric point, and dried under vacuum at 60 ± 1°C for 36 hours prior to analysis. The NaOH or HCl used to adjust the pH were reagent grade.

When a multiple stage extraction system was studied, the residue was resuspended in fresh solvent up to the same volume as that determined for the suspension of the previous stage.

RESULTS

The percent composition of the Jack bean seed, hull and meat, are shown in Table 1. As can be seen, the meat forms the larger percentage (85.63%) of the whole seed, being particularly rich in protein (38.65%) and starch (29.91%). In fact, the meat represents 87% and 96% of the total starch and protein content of the whole seed. On the other hand, the hull, representing 14.37% of the whole seed, has a high crude fiber content (61.20%) which represents 82% of the fiber content of the whole seed.

The results in growth, PER and mortality obtained for raw and cooked Jack bean seeds are summarized in Table 2. These findings indicate that althought the raw Jack bean produced negligible weight gain and PER values (2 ± 3 and 0.10 ± 0.34, respectively),

TABLE 1
PERCENT COMPOSITION OF THE JACK BEAN SEED, HULL AND MEAT
(Dry basis)

Component	Whole seed	Hull	Meat
Ether extract	3.49	2.31	4.61
Crude fiber	10.93	61.20	2.25
Protein (N x 6.25)	34.77	5.08	38.65
Ash	3.02	3.35	3.07
Nitrogen free extract	47.79	28.06	51.42
Starch	29.53	--	29.91
Total soluble sugars*	4.07	0.98	3.55
Percentage of whole seed	--	14.37	85.63

* Expressed as glucose.

it did not alter the mortality rate. A statisticaly significant ($P<0.01$) increase in both weight gain and PER values was obtained by cooking the Jack bean prior to the biological evaluation of its protein. Hewever, both the weight gain and PER values of the cooked legume were significantly lower ($P < 0.01$) than those reached with casein.

The results obtained through the protein-starch extraction method, as carried out at 25°C, are shown in Figure 1. In this case the meal:solvent ratio used to perform the extraction was 3:100. Deionized water served as solvent. As the data reveal, 87% of the meat nitrogen is extracted through a triple-stage extraction system. Approximately 90% of the extracted protein (or 79% of the original meat protein) is recovered through isoelectric point precipitation at pH 4.9 (4). It was found that the protein isolate, after being washed and dried, had around 90% protein content. Of the original starch, 95% was found in the final residue. The data indicate that the starch recovered has 49% of impurities;

TABLE 2
PERFORMANCE OF RATS FED RAW AND COOKED JACK BEAN MEAL*

Jack bean fed	Protein in diet (%)	Weight gain	Protein efficiency ratio	Mortality
Raw	13.1	2 ± 3**	0.10 ± 0.34	0/6
Cooked	13.3	40 ± 12	1.28 ± 0.24	0/6
Casein	13.2	137 ± 16	2.79 ± 0.27	0/6

* Cooked at 15 psig (121°C) for 20 minutes.

** Standard deviation of the mean

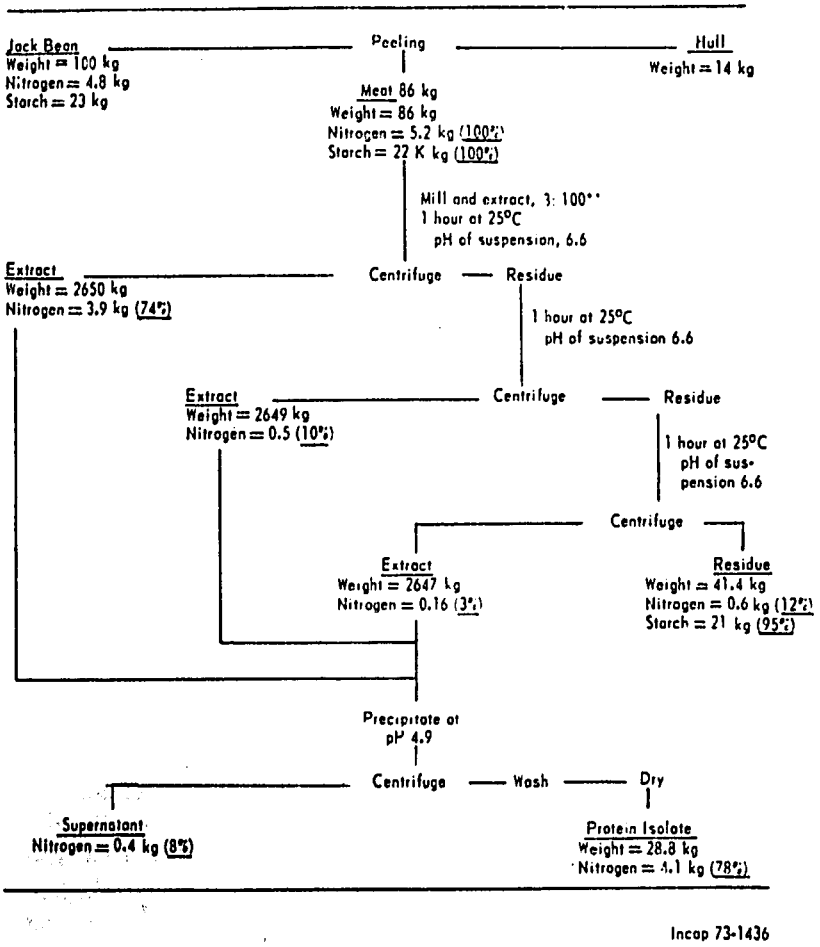
however, these can be eliminated easily by succesive washings and filtrations through cheesecloth, as described for the Mungo bean process (8).

Figure 2 shows the results obtained when the protein-starch extraction was performed at 40°C. The solvent and the meal: solvent ratio used were the same as those employed at 25°C. Results indicate that, in this case, 90% of the meat nitrogen can be extracted throught a double-stage extraction system. The percentage of protein and starch recovery and the protein content of the isolate were very similar to those obtained when using an extraction temperature of 25°C.

Increase of the extraction pH to 8.0 when carrying out the protein extraction at 40°C, raised the efficiency to 87% in a single-stage extraction. However, the use of such pH is not recommended since it could cause some damage to the isolated starch. The veracity of this possibility is still under investigation. When protein extraction was carried out at the pH of the aqueous suspension (6.6) at 40°C but using a meal:water ratio of 6:100, the extraction yield decreased to 40% and to 10% in the first and second stages, respectively. This indicates again the inverse relationship previously found between the protein extraction yields and the meal:solvent ratios in the case of the Jack bean (4).

Table 3 shows the essential amino acid profiles found for the Jack bean and for the Jack bean protein isolate obtained from the double-stage extraction, as carried out at 40°C (see Fig. 2). These essential amino acid profiles are compared with those reported for

the soy protein isolate "Promine" (15) and with the FAO protein reference pattern (16). As can be appreciated, the Jack bean protein isolate presents a higher essential amino acid content than both the original Jack bean and the soy protein isolate, and a higher lysine content than the original Jack bean. In all three protein sources reported, the total sulfur amino acids were the most limiting essential amino acids. The chemical score calculated against the



Incap 73-1436

Figure 1. Balance of materials from the protein-starch extraction carried out at 25°C *

* Figures in parenthesis represent percentages of meat nitrogen and starch.

** Meal:solvent ratio.

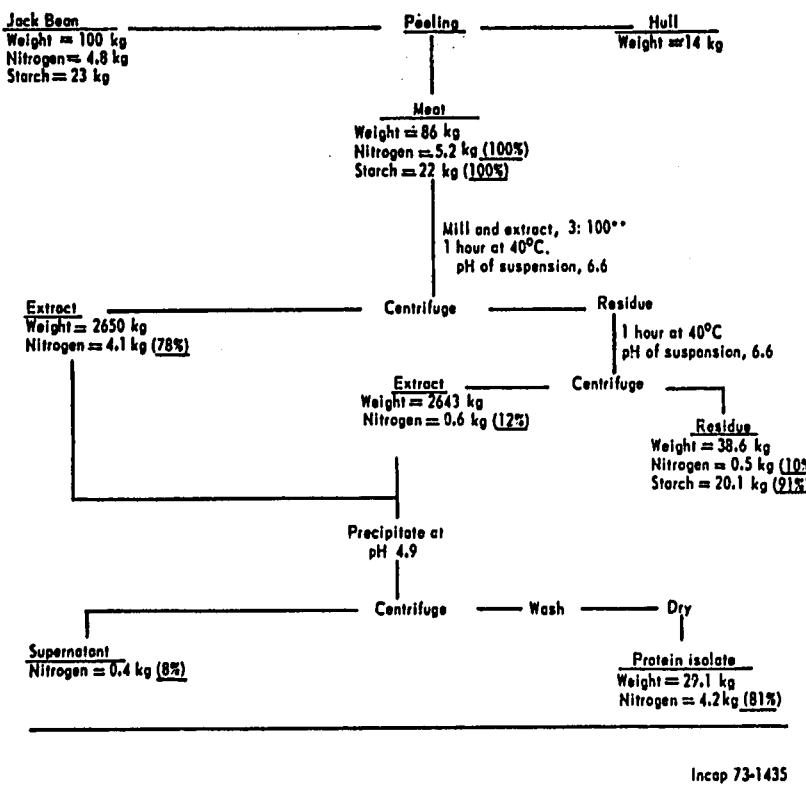


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Figure 2. Balance of materials from the protein-starch extraction carried out at 40°C *

* Figures in parenthesis represent percentages of meat nitrogen and starch.

** Meal:solvent ratio.

FAO protein reference pattern cited was 25.5 for the Jack bean, 29.2 for the protein isolate of this legume, and 38.5 for the soy protein isolate (Promine).

It should be noted that although the essential amino acid profiles of the Jack bean and soy protein isolates are very similar, the latter has a lower leucine/isoleucine ratio (1.6) than the former (2.2), a fact that favors the amino acid balance of the soy protein isolate. The Jack bean seed, on the other hand, presented the larger leucine/isoleucine ratio (2.4) when compared with that obtained for the FAO protein reference pattern (1.1).

TABLE 3

ESSENTIAL AMINO ACID PROFILES OF THE JACK BEAN MEAL, JACK BEAN AND SOY PROTEIN ISOLATES, AND FAO PROTEIN REFERENCE PATTERN (g/16 g N)

Amino acid	Jack bean meal	Jack bean protein isolate	Soy protein isolate (Promine)*	FAO protein** reference pattern
Isoleucine	3.4	4.9	5.0	4.3
Leucine	8.2	10.7	7.9	4.9
Lysine	6.0	6.3	6.3	4.3
Methionine	1.1	0.8	1.1	4.3
Cystine	0.2	0.9	1.0	
Phenylalanine	4.5	5.8	5.5	5.8
Tyrosine	4.1	3.6	3.8	
Threonine	4.3	3.4	3.7	2.9
Tryptophan	1.3	1.5	1.3	1.4
Valine	5.1	5.6	5.2	4.3

* Taken from Smith and Circle (15).
** Taken from FAO (16).

DISCUSSION

It is evident that the Jack bean has a larger protein content (Table 1) than most of the other legume seeds studied (12, 17). This characteristic favors the use of the Jack bean as raw material for processes involving protein extraction, since it would render a higher extraction yield. The starch content values obtained for this legume are very similar to those notified for other legume seeds (5, 6).

Since both components (protein and starch) are concentrated mainly in the meat (Table 1), the authors consider that using only this part of the seed for the protein-starch extraction does not affect the total yield. Furthermore, the initial elimination of the hull, facilitates the final purification of the starch.

The biological data obtained (Table 2) indicate that although the Jack bean contains growth-inhibiting factors (2, 3), these may be at lower concentrations than in the common bean *(Phaseolus vulgaris)*. Apparently this is true, since the raw Jack bean did not cause mortality when fed to rats, while the raw common bean has been reported to produce high mortality rates when fed to rats at similar or lower protein levels (18).

The above-mentioned observation is of interest, as it would indicate that, most probably, a mild short-heat treatment would be enough to destroy completely all the growth-inhibiting factors that could be present in the final protein isolate.

The low weight gain and PER values obtained with the Jack bean proteins when compared to the casein standard protein used (Table 2), is explained by the low concentration of sulfur amino acids and low chemical score found for the proteins of this legume seed (Table 3). On the other hand, from the chemical standpoint, the protein isolate is of a better quality, since it presents a higher concentration of total sulfur amino acids (Table 3).

It is of interest to note that the soy protein isolate, although chemically of a better quality than the Jack bean and its protein isolate, presents a lower total sulfur amino acid content than the soy bean (15), thus indicating that, in this case, the isolation process degrades the quality of the soy proteins.

Data in Figures 1 and 2 reveal that, evidently, the temperature has a beneficial effect on the protein extraction yield. This beneficial effect for the Jack bean protein extraction has also been informed by other authors (4).

From the extraction results obtained when using a temperature of 25°C (Fig. 1), we consider it adequate to recommend a three stage extraction system at 25°C, rather than a two-stage extraction system at 40°C (Fig. 2). This recommendation is based on the fact that the latter presents an efficiency (90%) similar to that of the former (87%), with advantage that it practically does not require any heat, since extraction is carried out at room temperature (25°C).

It has been reported that the Jack bean proteins are highly soluble at basic pH units (4). However, it is possible that the use of pH values higher than 7.5 could damage the starch, thus sacrificing its quality and probably also the starch recovery yields. The validity of such possibility is now under active investigation in our laboratories.

From the economic viewpoint, it is considered that a combined protein-starch extraction is more feasible than protein extraction alone, since it would yield two final products. The Guatemalan average starch price at the moment is $ 20 (U.S.) per 100 lb. Thus, the starch could absorb, at least in part, the total extraction costs.

Both the biological and functional characteristics of the extracted proteins are presently being investigated. However, since these are proteins in their natural state, not subjected to heat treatment either prior or during the extraction process, we believe that their functional properties would be similar to or better than those repor-

ted for the soy bean protein isolates. The latter are obtained from a residual cake derived of an oil extraction process (19).

The authors consider that the findings reported herein show that the Jack bean, a legume presently consumed in negligible quantities as a food item, but that grows easily producing high yields (1), could be used in the production of protein isolates and starch. The protein isolates, in turn, could be utilized in the production of either a texturized food item or several conventional food formulations.

SUMMARY

A protein-starch extraction process, applicable to Jack bean (Canavalia ensiformis), is described. A triple-stage extraction system using a 100:3 solvent: meal ratio, and a pH of 6.6 for one hour at 25°C, resulted in 87% protein and 95% starch extraction efficiencies while at 40°C, 90% protein and 91% starch efficiencies were obtained with a double-stage extraction system.

Amino acid analyses revealed that the Jack bean protein isolate had a higher essential amino acid content than either the Jack bean seed or a soy protein isolate, and a higher lysine content than the Jack bean seed. Total sulfur amino acids were in all cases the most limiting ones. The protein score for this legume, and for the Jack bean and soy protein isolates was 25.5, 29.2 and 38.5, respectively.

Although feeding raw Jack bean meal to rats resulted in negligible weight gains and protein efficiency ratio (PER), no mortality was observed. For this reason it is concluded that growth-inhibiting factors may be present in the Jack bean in lower concentrations than in the common bean (Phaseolus vulgaris). As expected, Jack bean protein quality was improved by cooking.

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Nutritional evaluation of protein concentrates from field peas (Pisum sativum) and faba beans (Vicia faba)

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INTRODUCTION

Field peas *(Pisum sativum)* and faba beans *(Vicia faba* L. minor) may be grown successfully in regions not well suited to the production of soybeans or other important food legumes. They also differ in not being regarded as an oil seed, which may sometimes be an advantage in terms of processing and nutritional usefulness.

Both field peas and faba beans have been analyzed and found to be relatively rich in protein content and in lysine but their proteins tend to be deficient in methionine (6, 8, 12). However they appear to complement the common cereals like wheat and rice reasonably well according to tests with rats (13).

Modern technology permits the fractionation of seed material like peas and beans into starch-rich and protein concentrate fractions thus making available concentrates containing upwards of 60% crude protein (%N x 6.25) for use in human or animal foods. This report pertains to the nutritional evaluation of such protein concentrates.

MATERIALS AND METHODS

Preparation of pea protein concentrate

The pea protein concentrate (PPC) was prepared by aqueous alkali extraction of pea flour. Field peas were split in a coffee mill and the hulls removed in a fanning mill. The split peas were ground further in a coffee mill to 100-mesh flour which contained

on analysis 24.5% crude protein, N x 6.25. A weight of 1.82 kg of this flour was stirred for 15 min with 13.64 liters of water to which 18 g of CaO had been added. The pH of the final slurry was 9.3. Starch was removed from the slurry in a solid bowl centrifuge 30 cm in diameter, operated at 2500 rpm. The supernatant solution was dried without neutralization on a double drum drier (30 cm diameter by 46 cm wide) at atmospheric pressure. Steam pressure to the drums was 40 psi (2.81 kg/cm^2). The yield of the dried protein concentrate was 36% of the initial pea flour weight and it had a protein content of 60% on a dry basis. The dried starch fraction had a protein content of 4.3%. (In a subsequent test the protein content of the starch was reduced to 1.7% by an additional washing with 13.64 liters of water containing 18 g of CaO).

Feeding trials with PPC

PPC, with and without supplementary methionine, was compared with fish protein concentrate (FPC) (courtesy Viobin Corporation, Monticello, Ill.), defatted whole egg (Nutritional Biochemicals Inc., Cleveland, Ohio) and casein plus methionine in feeding trials with mice. Each product was used as the only protein source in diets containing four protein (N x 6.25) levels, namely, 10, 15, 20, and 25% of the dietary dry matter.

Protein (N x 6.25) contents of diet ingredients and feces were determined by standard Kjeldahl procedures. Heat of combustion values were derived with an oxygen bomb calorimeter. Amino acids (excluding tryptophan) were obtained on PPC and FPC using a Technicon amino acid analyzer. Calcium and phosphorus were determined according to AOAC procedures (2), except that calcium readings were taken with an atomic absorption spectrophotometer.

The diets for all protein sources were formulated as shown in Table 1 for the PPC-plus-methionine diets. Variations in the protein content of the protein source were accommodated by proportional adjustments in the amounts of starch, glucose (provided as cerelose; a hydrolysis product of corn starch, largely glucose) and sucrose, which were prepared as a premix.

Weanling male mice of Carworth Farms Nº 1 origin and weighing 10-12 g initially were fed individually *ad libitum* for 14 days in wire-bottomed metal cages.

Five replicates, each of 20 mice, were used. Chromic oxide (Cr_2O_3, 0.5%, Table 1) was included in the diets. Feces were

collected during the growth test and pooled within diets to permit calculation of average digestibility coefficients based on five mice per diet. At the conclusion of the 14 day growth test the mice were killed, cleaned of gastrointestinal tract contents and analyzed for water, protein, fat and ash to allow energy and protein utilization studies.

The body composition was determined by first drying the digesta-free body *in vacuo* at 80C overnight, to determine water content. The dry matter was then coarsely ground in a high speed homogenizer, transferred quantitatively into a stainless steel (Swedish) tube and extracted with 2:1 chloroform:methanol to determine the fat content (15). The fat and residue were separated by means of a sintered glass funnel and repeated washings with solvent. The fat was subsequently weighed after evaporation of the solvent.

Heats of combustion were obtained, with the aid of a bomb calorimeter, on pooled samples of the extracted fat and on the

TABLE 1
FORMULAS FOR DIETS CONTAINING PEA PROTEIN CONCENTRATE (PPC) SUPPLEMENTED WITH METHIONINE

Ingredient	Protein in diet dry matter, %			
	10%	15%	20%	25%
Corn starch	33.07	28.48	23.89	19.80
Glucose	18.04	15.54	13.03	10.53
Sucrose	9.02	7.77	6.52	5.26
Cellulose	8.00	8.00	8.00	8.00
Lard*	2.50	2.50	2.50	2.50
Rapeseed oil*	2.50	2.50	2.50	2.50
Vitamins†	5.00	5.00	5.00	5.00
Minerals‡	4.50	4.50	4.50	4.50
Oxytetracycline	0.175	0.175	0.175	0.175
PPC	16.77	25.16	33.54	41.93
Methionine (1-)	0.42	0.38	0.34	0.30

*Lard and rapeseed oil were premixed with 0.25% ethoxyquin antioxidant.
†The vitamin premix contained 0.01 g thiamin, 0.02 g riboflavin, 0.10 g pantothenic acid, 0.10 g niacin, 0.002 g biotin, 0.01 g pyridoxine, 0.05 g cobalamin (0.1%), 0.005 g folacin, 0.04 g inositol, 8.00 g choline, 0.03 g vitamin A (325,000 IU/g), 0.0125 g vitamin D_3 (200,000 IU/g), 1.00 g vitamin E (27.5 IU/g), 0.40 g menadione and 90.5 g corn starch to 100 g.
‡The mineral premix contained 4.0 g iodized NaCl, 68.7 g $CaHPO_4$, 13.6 g $KHCO_3$, 1.8 g MgO, 0.3 g $MnSO_4.H_2O$ 0.3 g $FeSO_4.2H_2O$, 0.1 g ZnO and 11.1 g Cr_2O_3 per 100 g.
§A premix to provide 20 ppm in the diet.

residue in order to assign energy values to body composition data. Protein determinations were made on each residue, leaving ash to be determined by difference. Similar composition data were obtained for typical initial-weight mice in order to assess the nature of body growth during the feeding trials.

The data were subjected to analysis of variance and Duncan's multiple range tests (7).

The basal diet used for the evaluation of the faba bean concentrate (FBPC) contained 5% casein, replacing starch, but was similar otherwise. The FBPC diets were tested at dietary protein levels of 8, 12 and 16%. The same procedures were also followed in regard to the allotment of animals for the growth trials and the analysis of samples. However the digestibility tests were conducted using adult mice in tests involving three days adjustment period followed by four days of fecal collection, using specially designed cages for minimizing feed and urine contamination of feces.

RESULTS AND DISCUSSION

PPC Experiment

Nutrient content of PPC.

PPC proved to be lower than FPC in protein, energy, calcium, and phosphorus contents (Table 2). It was also lower in the levels of the various essential amino acids with the exception of arginine, as might be expected because of the difference in protein levels. However, a comparison of amino acids as percentages of the protein in the two products revealed a remarkable similarity, with perhaps two exceptions. PPC is notably deficient in methionine but somewhat richer in cystine. These results indicate that PPC appropriately supplemented with methionine might equal FPC in terms of protein quality.

Growth and feed utilization.

Considering the average gains observed for protein sources (Table 3), it is evident the mice fed PPC without added methionine gained less than those fed any other diet. However, there were significant effects of protein levels as well as interactions between levels and sources; consequently, more meaningful comparisons can be made within levels and within protein sources.

The weight gains with PPC diets (Table 3, protein sources x levels) were lower at 10% protein than at higher levels, but even at the 25% level PPC was inferior to either egg or casein.

When PPC was supplemented with methionine, marked improvements were obtained at the 15, 20, and 25% levels but not at 10%. Possible reasons for the latter observation will be given subsequently.

Feed intakes were not significantly different among the diets, but the amount of feed required per unit of weight gain indicated that methionine-supplemented PPC was equal to FPC, egg or casein and markedly superior to the unsupplemented PPC.

Energy Utilization

The gross calories of PPC were about 89% digestible and of the same order as the coefficients of energy digestibility for FPC, egg and casein (Table 3). There appeared to be a slight depression in digestibility associated with methionine supplementation, as was also found in studies with peas fed to pigs (3).

TABLE 2
COMPOSITION OF PEA PROTEIN CONCENTRATE (PPC) AND FISH PROTEIN CONCENTRATE (FPC). DRY MATTER BASIS

Composition of pea protein concentrate (PPC) and fish protein concentrate (FPC). Dry matter basis.

			Amino acids, g/16gN	
Nutrient	PPC	FPC	PPC	FPC
Protein (N x 6.25)%	59.62	85.45		
Arginine, %	6.14	5.88	10.31	6.88
Histidine, %	1.56	1.61	2.64	1.88
Isoleucine, %	2.41	3.60	4.04	4.21
Leucine, %	4.65	7.03	7.80	8.23
Lysine, %	4.69	6.67	7.88	7.80
Methionine, %	0.58	2.62	0.98	3.07
Tryptophan, %	0.80*	0.83†	3.30*	0.97†
Phenylalanine, %	3.02	3.51	5.08	4.11
Valine, %	2.69	4.06	4.51	4.75
Threonine, %	2.55	3.66	4.28	4.28
Cystine, %	0.96	1.02	1.59	1.19
Calcium, %	0.54	3.78‡		
Phosphorus, %	0.77	2.93‡		
Gross energy, kcal/g	4.77	5.57		

* Block and Bolling (4)

† Stillings (14)

‡ U.S. Dept. Int. (16)

On the basis of graphic plots of energy digestibility coefficients against levels of protein in the diets, it was calculated that the energy components of the protein sources *per se* had the following digestion coefficients: PPC 82, PPC + methionine 78, FPC and egg 89, and casein 90%.

Energy gained during the 14 day test was of similar magnitude for all levels of unsupplemented PPC, indicating that the slower gaining mice fed 10% PPC were fatter. This was confirmed by the body analysis which showed 10.7, 8.5, 8.5 and 9.1% fat in the digesta-free bodies of mice fed 10, 15, 20, and 25% PPC, respectively.

TABLE 3

EFFECTS OF PROTEIN SOURCES AND LEVELS ON WEIGHT GAINS, FEED CONSUMPTION AND UTILIZATION OF ENERGY

Treatment	Weight gain g	Feed intake g	Feed/ gain ratio	Digestibility of energy %	DE intake kcal	Energy gain kcal
Protein sources						
PPC	6.5	46	7.0	88.9	176	13.4
PPC + meth.	10.5	50	4.8	87.0	186	23.3
FPC	9.4	50	5.3	90.0	198	20.3
Egg	10.4	48	4.6	90.1	193	21.5
Casein + meth.	11.2	50	4.4	88.7	196	27.8
LSR Min.	1.9	NS*			NS	4.2
Max.	2.1					4.6
Protein levels %						
10	6.7	50	7.5	89.4	188	15.3
15	10.0	50	5.0	89.4	192	21.7
20	10.8	49	4.5	88.5	191	24.3
25	10.9	47	4.3	88.6	188	25.8
LSR Min.	1.7	NS			NS	3.8
Max.	1.9					4.1
Protein sources x levels						
PPC 10%	5.0	47	9.4	90.4	178	12.7
15%	6.4	47	7.3	89.3	180	12.8
20%	7.9	47	6.0	88.2	180	14.4
25%	6.8	42	6.4	87.8	164	13.8
PPC + meth						
10%	4.5	50	11.1	88.1	185	11.9
15%	12.9	52	4.0	88.3	195	28.1
20%	12.7	51	4.0	85.5	190	26.7
25%	11.7	47	4.0	86.3	177	26.7
FPC 10%	7.8	52	6.6	90.2	199	17.6
15%	9.5	52	5.5	89.9	205	20.0
20%	10.1	48	4.8	90.1	194	22.9
25%	10.2	48	4.7	90.0	195	20.7
Egg 10%	8.2	49	6.0	90.5	186	16.6
15%	11.1	49	4.4	89.9	193	22.2
20%	10.5	46	4.4	90.1	188	23.8
25%	11.9	50	4.2	89.9	206	23.6
Casein + meth.						
10%	7.8	52	6.6	87.7	195	17.8
15%	10.0	48	4.8	89.5	189	25.6
20%	13.0	51	3.9	88.6	204	33.6
25%	14.1	48	4.4	89.0	196	34.1
LSR Min.	2.9	NS			NS	8.4
Max.	3.1					9.2
Error mean square (80 D.F.)	5.21	48.93			738.23	25.4

TABLE 4
EFFECTS OF PROTEIN SOURCES AND LEVELS ON PROTEIN UTILIZATION

	Digestibility of protein, apparent %	Biological value	
		Apparent	True
Protein sources			
PPC	85.4	19	30
PPC + methionine	85.0	24	35
FPC	84.9	27	41
Egg	85.8	31	42
Casein + methionine	87.5	29	40
LSR Min.		4.9	6.2
Max.		5.4	6.8
Protein levels, %			
10	83.8	29	46
15	86.4	30	44
20	87.1	25	34
25	86.8	19	27
LSR Min.		4.4	5.6
Max.		4.8	6.1
Protein levels x sources			
10% PPC	86.3	23	40
PPC + meth.	83.7	12	29
FPC	80.1	36	54
Egg	81.6	41	58
Casein + meth	83.1	34	50
15% PPC	88.0	21	33
PPC + meth	85.2	34	47
FPC	85.3	29	51
Egg	85.7	35	47
Casein + meth.	87.6	31	43
20% PPC	87.7	19	28
PPC + meth.	84.0	28	37
FPC	86.6	24	34
Egg	87.0	26	36
Casein + meth	90.1	27	34
25% PPC	87.8	13	21
PPC + meth.	87.1	21	29
FPC	87.8	18	26
Egg	88.9	21	28
Casein + meth.	89.3	25	32
LSR Min.	9.8	9.8	12.4
Max.	10.7	10.7	13.6
Error mean squares (80 D.F.)		34.46	55.20

When PPC was supplemented with methionine there were marked improvements ($P < 0.05$) in energy gain. At or above 15% PPC the calorie gains equalled those obtained with diets containing FPC, egg or casein.

Protein Utilization

The protein in PPC was about 87% digestible and therefore as well digested as any of the other sources (Table 4), but methionine addition resulted in some depression of protein digestibility at all levels of dietary protein with perhaps less effect at 25% protein.

It was possible to estimate the metabolic fecal nitrogen (MFN) excretion in this experiment by extrapolating to a zero-protein diet, and a value of 0.15 g MFN/100 g dietary dry matter was obtained for diets containing casein, egg or FPC. From this the true digestibility coefficients for these three proteins were found to be 93, 91, and 91%, respectively. The MFN values derived for PPC and PPC + methionine diets were lower than for the other diets, indicating that the presence of PPC in the diet reduced the endogenous nitrogen losses from the gastrointestinal tract. This finding may be related to the observations by Borc and Bakeeva (5) that pea meal inhibited the secretion of gastric juice and reduced the enzyme content in the stomach of the growing pig. Bell and Wilson (3) reported non-linear effects on digestion coefficients when 0, 20, and 40% peas were used in pig rations, and a favorable effect of peas on digestibility of the basal ration. Subject to further investigation of this apparent characteristic of peas, the true digestibility of pea protein appears to be about 88%, slightly lower than for the other protein sources.

Protein quality was assessed in terms of biological value (BV), using both apparent BV and values calculated by the Thomas-Mitchell method (9). From a comparison of protein sources within levels it is evident that PPC was inferior in quality to the other sources at the 10% level, and it is also obvious that supplementation with methionine at this protein level was deleterious.

When 15% protein levels were used PPC was still inferior to FPC and egg, and possibly to casein, but methionine addition was beneficial and rendered PPC equal to the other proteins. However, at 20% protein the biological values were being depressed more markedly by the effects of higher protein levels and the effect of methionine was reduced. With 25% protein there were no differences detectable in BV.

A comparison of the amino acid composition of the various diets with the dietary requirements of the mouse is of value in explaining the varying responses to supplementary methionine. Even though egg and casein are commonly used as reference

proteins in protein quality studies, their amino acid complements do not coincide with the pattern of known dietary requirements for growth. Unfortunately, the amino acid requirements of the laboratory mouse are not known as accurately as those of the rat (11), but these two animal species are considered to have rather similar requirements. On this basis the data in Table 5 provide some insight into the methionine responses.

When 10% dietary protein was used, methionine was severely deficient and would be the first limiting amino acid. However, histidine, phenylalanine and valine were also present at levels significantly below requirements, and these would prevent an effective response to supplementary methionine. It is probable that the sulfur-containing amino acids (methionine and cystine) in these PPC diets provided less than 35% compared with 56 to 65% of requirements for the other three major limiting amino acids in PPC. The addition of sufficient methionine to bring the level to 100% of requirement level may have further aggravated the amino acid balance and resulted in methionine toxicity (1).

TABLE 5

AMINO ACID CONTENTS OF DIETS RELATIVE TO THE REQUIREMENT LEVELS OF AMINO ACIDS FOR THE GROWING RAT (AMINO ACID REQUIREMENT OF THE RAT = 100)

Amino acid	10% protein diets				15% protein diets			
	Egg*	FPC	Casein*	PPC	Egg	FPC	Casein	PPC
Arginine	310	290	185	501	465	440	290	775
Histidine	52	40	75	65	77	60	112	98
Isoleucine	120	72	130	80	186	108	188	120
Leucine	100	78	110	86	150	105	168	130
Lysine	61	67	81	79	91	100	120	118
Methionine	53	43	53	17	80	65	80	25
Phenylalanine	62	59	60	56	93	98	90	84
Threonine	98	74	90	86	146	103	134	128
Tryptophan	55	50	50	165	80	75	75	245
Valine	100	57	105	64	150	85	158	93

*From data by Block and Bolling (4) for egg and casein and for tryptophan in peas. Amino acids in FPC and PPC derived from Technicon amino acid analyzer, except for tryptophan.

At the 15% protein level it is evident that methionine was the only amino acid that was grossly deficient in PPC diets. Correction of this inadequacy by supplementation would then rank egg, FPC and casein in the position of having more severe amino acid limitations than methionine-supplemented PPC, assuming that no important differences exist in availability of amino acids.

It is concluded that PPC contains protein that is of slightly lower true digestibility than that of egg, FPC or casein, namely 88% versus 91 to 93%, and contains energy-yielding constituents that are about 80% digestible compared with 89 to 90% for the other protein sources. The protein quality of PPC was inferior on account of methionine deficiency, but equalled that of egg, FPC or casein when fed at a 15% protein level and supplemented with methionine. It is emphasized that the quality tests applied here were more demanding than in situations where the test protein would not be the sole source of dietary protein. Thus, the apparent shortage of methionine may be less important in many potential food use applications than occurred in these tests. Bell and Wilson (3) and Moran et al. (10) obtained satisfactory responses in swine and chicks, respectively, fed production diets containing ground field peas without supplementary methionine.

TABLE 6
GROWTH AND FEED UTILIZATION RESPONSES OF GROWING MICE FED DIETS CONTAINING FABA BEAN PROTEIN CONCENTRATE COMPARED WITH SOYBEAN MEAL

Protein source	Protein level in diet %	Weight gain g	Feed intake g	Feed/gain ratio
Soybean meal	8	7.2	52.8	7.1
	12	10.7	50.9	4.8
	16	13.2	54.1	4.1
FBPC	8	5.4	50.3	9.2
	12	8.3	50.6	6.1
	16	11.6	55.9	4.8

FBPC Experiment

Growth and feed utilization. The mice consumed the FBPC diets as readily as those containing soybean meal (Table 6) but their growth rates and efficiency of feed conversion were slightly inferior to soy-fed mice. The improvement in weight gains coincident with increasing levels of dietary protein was reflected in improved efficiency of conversion of gross dietary energy intake into body gains, but the efficiency was slightly poorer with FBPC diets (Table 7). PER values indicated no difference between soybean

ıeal and FBPC at the 8% dietary protein level but soy protein ıay have been superior at the two higher protein levels.

The amino acid composition of the protein in faba beans Table 8) indicates this protein being richer in arginine, leucine, ysine and possibly histidine than soybean protein, and somewhat ›wer in sulfur amino acids, methionine and cystine. Faba bean rotein resembles soybean protein in having essential amino acids ›mprising about 45% of its protein (N x 6.25) whereas pea rotein has about 30% essential amino acids.

TABLE 7
PROTEIN EFFICIENCY RATIOS AND EFFICIENCY OF ENERGY UTILIZATION

rotein source	Protein level in diet %	Gross energy intake kcal	Energy stored kcal	% of gross energy stored	PER
oybean meal	8	238	17	7	1.73
	12	230	23	10	1.79
	16	244	29	12	1.46
BPC	8	228	13	6	1.75
	12	230	17	7	1.46
	16	255	23	9	1.26

In order to examine the reasons for the failure of FBPC permit growth and feed utilization equal to those from soybean ets (Tables 6 and 7) the amino acid compositions of the diets ere compared with the dietary requirements for the growing t (Table 9). It is apparent that the 8% protein diet may have en deficient in eight amino acids, with methionine-cystine being e most seriously lacking. At 12% protein, methionine + cystine, ptophan and phenylalanine were deficient and at 16% protein ethionine + cystine was the only one lacking and the level is approximately half the requirement level. Thus, if the amino id requirements of rats and mice are similar, it would be pected that the 16% protein diet would result in subnormal rformance.

TABLE 8
AMINO ACID COMPOSITION OF THE PROTEIN IN FABA BEANS COMPARED WITH SOYBEAN PROTEIN

Amino acid	Faba bean*	Faba bean†	Soybean
		g/16gN	
Arginine	10.5	8.7	7.1
Histidine	3.8	2.5	2.6
Isoleucine	3.7	4.3	4.5
Leucine	8.4	7.9	7.3
Lysine	7.6	6.3	6.5
Methionine	1.0	0.8	1.3
Methionine + cystine	2.1	1.9	2.6
Tryptophan	--	1.0	1.2
Phenylalanine	3.5	4.2	4.8
Valine	4.4	5.0	4.8
Threonine	4.1	3.9	4.0
Cystine	1.1	1.1	1.3

*As determined in our laboratory with a Technicon amino acid analyzer

†Average of 3 published results: references 6, 6, 12

TABLE 9
AMINO ACID COMPOSITION OF THE FBPC DIETS COMPARED WITH THE AMINO ACID REQUIREMENTS OF THE GROWING RAT

	Rat req't.	% dietary protein		
		8	12	16
		% in diet		
Arginine	0.2	0.69	1.29	1.90
Histidine	0.4	*0.31**	0.53	0.74
Isoleucine	0.5	*0.45*	0.65	0.86
Leucine	0.8	0.83	1.30	1.76
Lysine	1.0	*0.71*	1.14	1.56
Methionine	0.6	*0.18*	*0.22*	*0.27*
Methionine + cystine		*0.25*	*0.37*	*0.48*
Tryptophan	0.2	*0.10*	*0.15*	0.21
Phenylalanine	0.7	*0.39*	*0.58*	0.77
Valine	0.7	*0.54*	0.78	1.01
Threonine	0.5	*0.39*	0.62	0.85

*Values italicized indicate dietary levels below those required.

Preliminary data on digestibility indicates that the protein and energy components of raw faba beans are slightly less digestible than the corresponding fractions in soybean meal. This would tend to increase the apparent amino acid deficiency. These studies on FBPC indicate that it resembles PPC in being relatively deficient in methionine (+ cystine) but PPC protein contained more tryptophan and phenylalnine. There were indications that FBPC was slightly less digestible than PPC.

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SUMMARY

Pea protein concentrate (PPC) prepared by aqueous alkali extraction and faba bean protein concentrate (FBPC) prepared by pin-milling followed by air classification of dehulled faba beans were analyzed for protein, energy and amino acid content and fed to mice in growth and digestibility trials. PPC (60% protein) with and without supplementary methionine was compared with fish protein concentrate, egg and casein+methionine. Each protein source was tested at dietary protein levels of 10, 15, 20 and 25%, using a protein-free basal diet. The FBPC (75% protein) was tested at dietary protein levels of 8, 12 and 16%, using a semi-purified basal diet containing 5% casein and was compared with soybean meal. Protein and energy utilization was determined from body composition. Digestibility coefficients were obtained by using 0.5% Cr_2O_3 in the diets as a chemical marker. Both PPC and FBPC contained less methionine per unit of protein than occours in egg or fish protein. The methionine deficiency was confirmed by the feeding trials. The true digestibility of PPC protein was 88%, compared with 91 to 93% for egg, fish or casein. PPC energy components were about 80% digestible, compared to 89 to 90% for the other sources. The digestibility of energy in FBPC was about 90% and its protein was about 90% truly digestible. Protein efficiency rations (PER) calculated from responses to the 8% protein diets were 1.75 and 1.73 gm. gain/gm. dietary protein for FBPC and soybean protein respectively. Both PPC and FBPC are well digested, and are good dietary sources of protein when the low methionine/cystine content is corrected by appropriate diet formulation.

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Utilization of beans and other pulses in the preparation protein-rich foods for human consumption

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INTRODUCTION

Pulses are one of the principal sources of proteins and calories for human beings. And certainly make up an important part of the foods eaten by all Latin American peoples.

Traditionally these pulses, belonging, to the genus *Phaseolus, Vicia, Pisum, Cicer. Cajanus, Dolichos, Vigna* and others, are prepared for eating by using simple home proceedures such as boiling, toasting, stewing, in soups, purees, fried patties made from the purees, and tortillas.

Foods made from soya beans *(Glycine maxima),* which is an oil-producing legume seed, are also eaten, but the proceedures for the preparation of these for human consumption are usually complex. However they are becoming increasingy important in the feeding of people.

This paper describes some experiments which were designed to formulate and develop protein-high foods made from beans, which could then be used to enrich other food-products that are poor in both the quantity and quality of their proteins, such as many breads and noodles.

MATERIALS AND METHODS USED

The Nutrition Department of the Universidad Agraria made some attempts at enriching noodles and bread with products made from beans and Lima beans *(Phaseolus vulgaris* and *Phaseolus unatus),* they are described in this paper.

The studies made on noodles were carried out in the pa laboratory, using a Parmegiana press and a drier with forced a

The bread-enriching experiments were carried out in t experimental bakery and the animal laboratory using a swe bread which was developed in the department, called "Camo Pan". Bacigalupo *et al* (1).

TABLE 1

SWEET POTATO BREAD (CAMOTE-PAN) FORMULA F-1
UNIVERSIDAD NACIONAL AGRARIA - LA MOLINA - PERU

	Kgs.	
Wheat flour	32.00	
Sweet potato puree	20.00	
Yeast	1.20	
Brown sugar	8.00	
Oleo margarine	4.50	
Salt	0.48	
Candied fruit (or raisins)	1.60	
Eggs		20 units
Water		5 liters
Vanilla		50 cc
Artificial coloring		10 g.

TABLE 2

CHEMICAL COMPOSITION AND P.E.R. OF SWEET POTATO BREAD

	%
Moisture	30.70
Protein	10.49
Fat	10.50
Fiber	1.18
Ash	1.88
Nitrogen free extract	45.24
Calories	307.14
P.E.R.	-0.01

This product is usually made of wheat flour, gelatinized sweet potatoe *(Hipomea batata)* in the amounts shown in Table 1. The chemical composition and nutritional value of the bread are shown in Table 2.

The acceptability of this bread has been excellent; this is evident considering the results of the tasting panel, Table 3, and by the demand of the general public for it.

Unfortunately, although this bread is a delicious and cheap source of calories, its protein content leaves much to be desired. Its protein efficiency ration is low. Thus the sweet potatoe bread made in this way could only aspire to being an economic asset to the country, as it cuts down on the need to import wheat, it could not, however, claim to relieve the protein defficiency in the diet.

RESULTS AND DISCUSSION

I. *Production of noodles enriched with beans*

Wheat and beans have complementary aminograms as wheat is low in lysine, but rich in sulfur amino acids, and there are many varieties of beans rich in lysine but poor in methionine and cystine.

It is possible to bring about a good combination of wheat and beans in various foods, but bread and noodles are particularly appropiate. Noodles and other "pastas" offer a series of advantages from the point of view of nutrition, technology and marketing. For one thing the production of noodles does not require very high temperatures and no sugar is incorporated. This helps to maintain the good quality of the protein. Furthermore noodles are easy to transport and store, and lastly, as noodles are prepared at home by boiling, this ensures that the protein quality is not spoiled. For these reasons noodles are particularly apt for being successfully enriched with protein concentrates, the risks of negative chemical reactions occurring are reduced and the nutritional quality of the product remains high.

Different varieties of *Phaseolus vulgaris* and *Phaseolus lunatus* (pallares) were used in the Universidad Agraria for the enriching of noodles, in concentrations of between 15 and 40% with excellent results from the technological and organoleptic points of view. Good quality noodles of different flavours and colours were produced which varied according to the chemical and physical properties of the varieties used and to the degree of enrichment.

TABLE 3
PHYSICAL AND ORGANOLEPTIC CHARACTERISTICS OF CAMOTE-PAN (SWEET POTATO BREAD)

1) Bread weight before baking, g	480
2) Bread weight after baking, g	430 aprox.
3) Bread volume, cc	990 aprox.
4) Crumb color *	97
5) Crumb structure *	95
6) Crust texture *	99
7) Aroma *	98
8) Flavor *	100
GRADE	VERY GOOD

* Average of 100 tasters

Scale:				
Very poor	50 points	Good	80-89	points
Poor	60- 69 points	Very good	90-99	points
Fair	70- 79 points	Excellent	100	points

II. *Production of "Camote Pan" enriched with bean based products*

The need for the sweet potato bread to contribute to an improvement in the area of protein consumption led to studies in the enrichment of this bread, which are described below.

A) *Experiments in incorporating sweetened pallares (Phaseolus lunatus) in the dough*

Among the various beans studied, pallares *(Phaseolus lunatus)* were selected because so far they have the highest PER among beans studied in Peru. Sweetened pallares beans were used to replace the candied fruit in the bread so as to increase the nutritional value without diminishing its acceptability. This idea was based furthermore on the local practice of eating "Frejoles Colados" (sweetened common beans), which are highly appreciated.

The sweetened pallares beans were prepared by making a strained purée of the beans and sweetening it with 50% "chancaca" a very dark unrefined sugar similar to panela and 25% fat and spices.

In the initial experiments the beans were incorporated directly into the dough, but the results were not satisfactory as the bread took on an unpleasant flavour and colour and did not develop sufficent volume or sponge, all these being characteristics essential to maintaining the attraction of the bread. But on the other hand, the protein value went up from 0.1 to 0.21, and 0.32 when 5 and 10% respectively of the sweetened bean were added. The protein content went up from 10.40% to 12.21%. This solution was obviously not satisfactory, particularly when the decrease in acceptability was taken into account.

The poor results obtained in this experiment can be explained as the reaction of the free functional groups of some amino acids in pallares, with the aldehydes of sugars. The reaction as seen by the dark colour of the sweet bean and the bread, probably lowered the availability of the lysine, arginine histidine, tyrosine and methionine, according to the work of Rhubenthaler et al (13) and Lowe (9).

The high concentration of sugar in the sweet potato bread does not favour the utilization of the protein in the digestive tract either as the rapid absorption of sucrose leaves part of the amino acids without the necessary amounts of calories necessary to favour protein synthesis, Bender (2), Krehl et al (6), Rose (11) and Sabiston et al (14).

With the object of seeing if the Maillard reaction between the sweetened pallares and the sweet potato bread could be lessened during processing, it was decided to incorporate the sweetened beans without mixing them in the dough, but by spreading them on the extended dough, thus when it was subsequently rolled up, the two layers remained physically separated during fermentation and baking. The results obtained were considerably better from the point of view of appearance and protein value, as may be seen in Table 4. This type of sweet potato bread was called "Swiss Roll" (pionono) because of it's appearance and it had a protein value 2½ times higher than that of the bread made with same amount of beans incorporated into the dough (Graph 1), probably because the sugar of the dough was not allowed to react with the protein of the sweetened beans. Obviously the reactions of the bean proteins with the sugars in the dough were more harmful to protein quality than those of sucrose and bean proteins, when the sweetened pallares beans was prepared. It is possible, according to studies of Euler, cited

by Summer and Somners (15) and William (16), that in the sweet potato dough sucrose was inverted by the action of yeast liberating glucose and fructose.

During preparation of the sweetened pallares beans sucrose was less hydrolized, since sucrose was not present. Other types of reactions, like margarine decomposition products and aminolysis caused by alpha or epsilon amino groups reacting with sucrose proposed by El-Nockrasky (5) could explain the moderate reduction of PER in the sweetened bean.

This step forward prompted some research into the effect of the formulation and preparation of the sweetened beans on its protein value.

B) *Studies on the effects of sugar, oil and drying temperature on the protein efficiency ratio of pallares (phaseolus lunatus) flour*

Table 5, shows the results of cooking peeled soaked beans in several different ways. One lot was cooked for 40 minutes at 95°C without the addition of sugar (chancaca) and were dried at different temperatues to observe the effect of bread-beaking on the cooked pallares protein. A temperature of 60°C was selected as a control and for experimental treatments 90°C and 210°C since 90°C is the temperature at which the crumb dehydrates during baking, and 210° is the temperature which the crust reaches and at which it dehydrates during baking. A second lot of pallares beans was cooked at 95° for 25 minutes, then the unrefined sugar (Chancaca) was added and it was cooked for a further 15 minutes al 95°. As in the previous case the beans were dried at 60°, 90° and 210°C.

TABLE 4

PROTEIN EFFICIENCY RATIO OF SWEET-POTATO BREAD (CAMOTE-PAN) ENRICHED WITH SWEETENED BEANS (PHASEOLUS LUNATUS) MIXED OR INSERTED IN THE DOUGH

	Casein	Camote-Pan(1)			
		Candied fruit 5%	Sweetened beans (2)		
			Mixed in the dough 5%	Mixed in the dough 10%	Inserted in the dough (in layers) 10%
1. Initial weight, g.	43.85	40.75	43.45	43.83	46.10
2. Final weight, g.	91.20	40.18	45.93	46.07	55.40
3. Weight gain, g.	47.35	-0.57	2.48	3.24	9.30
4. Feed consumption per animal, g.	162.40	92.75	99.03	98.91	120.53
5. % protein in the diet	10.89	9.00	10.22	10.18	9.24
6. PER	2.68	-0.10	0.28	0.32	0.84
7. PER % of casein	100	———	10.45	11.94	31.34
8. Duncan test (0.05)			———		

(1) 12% MS of sweet potatoes (cooked, peeled and mashed)

(2) Sweetened beans (ph. lunatus): 58% dry beans, 28% unrefined sugar, 14% Oleomargarine and species.

TABLE 5
INFLUENCE OF SUGAR, OIL AND DRYING TEMPERATURES ON THE PER OF PHASEOLUS LUNATUS (PALLARES FLOURS) (1)

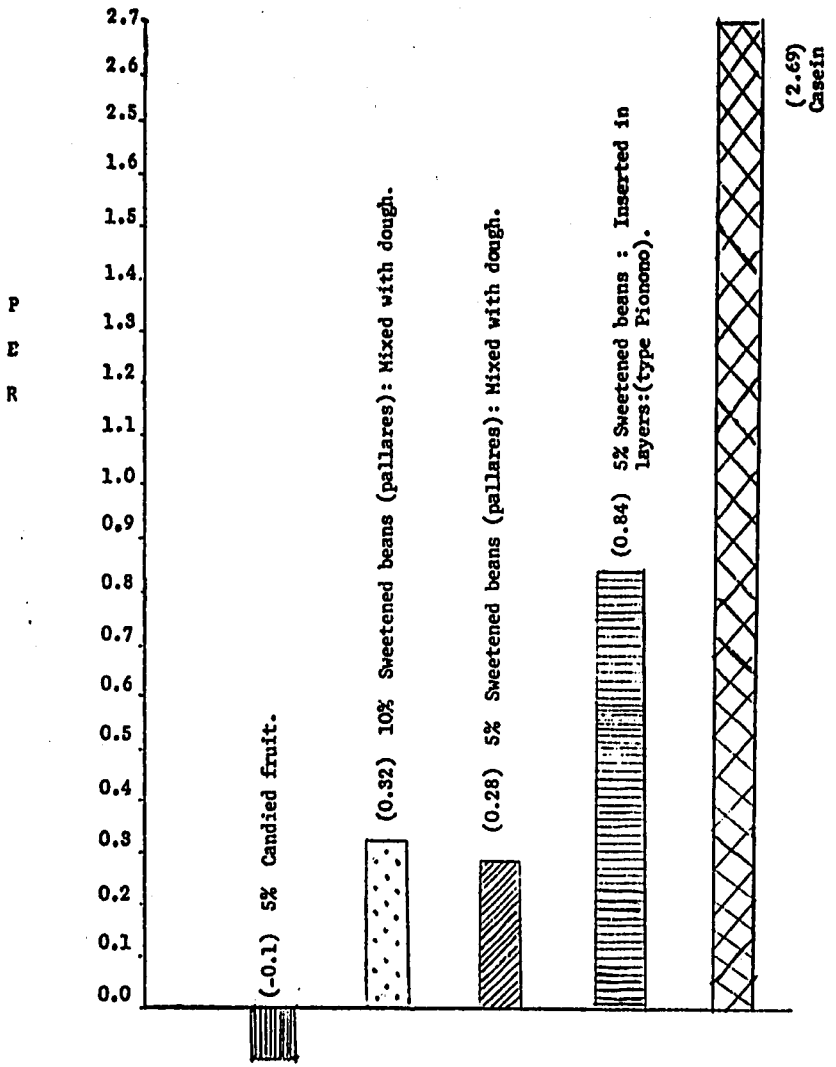
	Casein	Pallares[2] without sugar						Pallares[2] with sugar					
		60° x 24 hours		90° x 22 min.		210° x 22 min.		60° x 24 hours		90° x 22 min.		210° C x 22 min.	
		0	10% Oil	0	10% Oil	0	10% Oil	0	10% Oil	0	10% Oil	0	10% Oil
1. Initial weight, g.	43.85	45.16	45.59	43.96	43.17	43.56	43.10	45.22	43.75	42.87	44.30	45.58	44.30
2. Final weight, g.	91.20	77.44	77.28	70.14	85.91	70.40	91.90	59.58	71.33	60.93	51.27	59.06	58.85
3. Weight gain	47.35	32.28	31.69	26.18	42.74	26.84	48.80	14.34	27.64	18.06	6.97	13.48	14.55
4. Feed consumption/ animal, g.	162.40	163.9	144.27	146.75	240.95	154.71	248.45	127.10	199.63	160.16	160.84	124.66	181.25
5. % protein in the diet	10.89	10.00	10.80	10.49	10.02	10.30	10.31	10.42	9.24	9.79	9.79	9.79	10.04
6. PER	2.68	1.97	2.03	2.06	.1.77	1.68	1.91	1.08	1.50	1.15	0.44	1.10	0.80
7. PER % of casein	100	73.51	75.75	76.87	66.04	62.69	71.27	40.30	55.97	42.91	16.42	41.04	20.85

(1) Pallares (phaseoulus lunatus) soaked, peeled and cooked during 40 min. at 95° C and dried at different temperatures

(2) Pallares (phaseoulus lunatus) soaked, peeled, cooked during 25 min. at 95° C, then mexed with chancaca (a type of panela: unrefined sugar) and cooked again during 15 min. at 95° C. Finally dried at different temperatures.

GRAPH 1

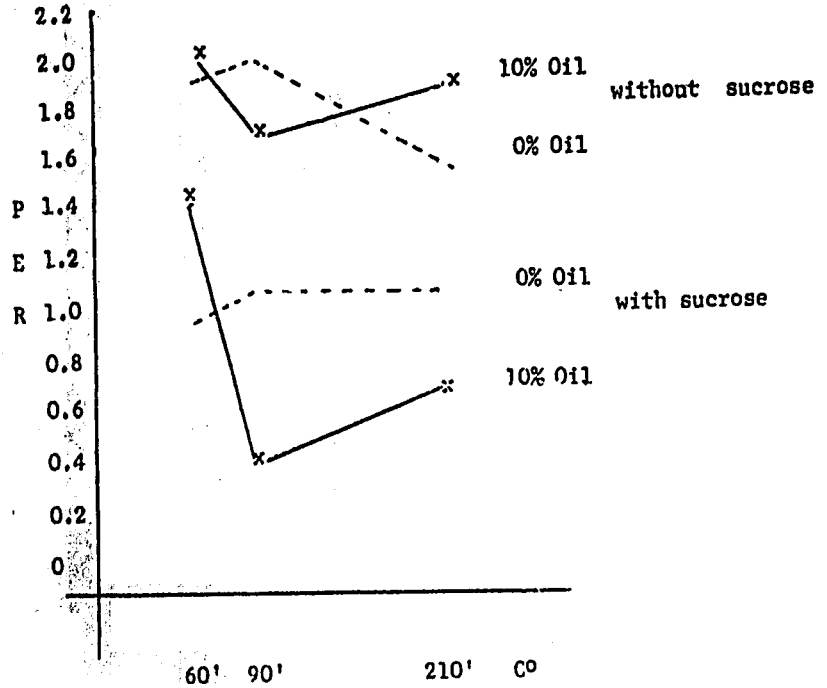
P.E.R. OF ENRICHED CAMOTE-PAN (P2) WITH SWEETENED BEANS (Phaseolus lunatus)



Graph 2, shows that adding sugar during the heating process had a very marked negative effect on the biological value of the bread. On the average the protein value was reduced by half. It was evident that when sugar was added the availability of amino acids was reduced, as much during the cooking at 95°C as during the drying, more so when higher temperatures were used.

GRAPH 2

INFLUENCE OF SUGAR, OIL AND DRYING TEMPERATURE ON THE P.E.R. OF PALLARES (Phaseolus lunatus) FLOURS



The worst results were obtained when the pallares beans were cooked with sugar and dried at 90°C and at 210°C, which agreed with the findings of Rosenberg et al (12), Bender (2) and Cleg (3), Ellis (4) and Hodge (7) explains that in similar conditions the terminal amino group of lysine, can react with the products of secondary decomposition which are formed when fats are in the process of oxidation. On the other hand, the work of Lea et al (8) indicates even more clearly the effect of fats by showing that at temperatures under 100° C lysine is lost with the autoxidizing fats but that at higher temperatures (115-130°C) the loss is

apparently independent of the presence of fats. These observations could well explain why the PER was lower when the drying in our experiment was done at 90°C and 10% of oil was used whether or not there was sugar added to the beans. It also shows why the lots that contained no oil had a good PER when the beans were dried at 90°C.

Furthermore it should be mentioned that in the pallares flours studied there must have been some destruction of proteins and amino acids because of the high temperatures used. Mohamad and Conrat (10) show that when unsweetened bread is baked, and the temperature of the crust by caramelisation, a reduction in the protein content of 10%, Ketrovich and Paramasiva, quoted by Rubenthaler et al (13), found that in wheat and rye bread there was a significant reduction in free amino acids in the crust, compared with those found in the crumb and dough. This reduction is also attributed to the participation of the free amino acids in the formation of melanoidins.

It may be considered certain that the destruction of proteins and amino acids in the sweet potato bread must have been very notable due to the presence of high quantities of reducing sugars during the baking process.

C) *Studies on protein pellets made from pallares (Phaseolus lunatus)*

When the negative effect of sugar and oil on the nutritive value of pallares protein was made evident, it became necessary to propose a new and better use for these proteins, taking the following consideration into account:

1. That the product should have none, or very little sugar and/or oil.
2. That it should be easily incorporated but not mixed with the dough, to avoid the Maillard reaction.
3. That it should have good functional properties in relation to the crumb of the bread.
4. That it should not affect the volume or the texture of the bread.
5. That the final product should be pleasant-tasting.

A pellet of appropriate texture was considered the best way to resolve the problem, and after several attemps pellets were prepared and a series of formulas were made, basically from pallares flour, potatoe or corn starch, fat, salt and flavouring.

TABLE 6
PALLARES PROTEIN SHOT FORMULAS (1)

Ingredients	101 %	102 %	103 Salted %	104 Strawbery %	105 Coffee %	106 Chocolate %	107 Banana %	108 Cranberry %
Cooked ph. Lunatus Flour(2)	54.70	73.90	63.42	61.22	67.41	62.24	60.60	71.09
Corn of Potato Starch	9.00	9.20	———	7.14	11.25	10.32	7.07	8.29
Margarine	———	5.26	19.02	4.08	4.64	5.18	4.04	4.73
Salt	2.70	1.33	10.57	2.04	2.24	2.07	3.04	2.40
Black pepper	1.70	5.26	5.30	1.02	———	———	1.01	1.18
Eggs	16.00	———	1.69	9.13	10.10	9.33	9.09	10.66
Garlic	1.58	———	———	———	———	———	———	———
Artificial coloring	0.03	0.05	———	0.04	2.24	7.20	———	0.47
Onions	14.00	———	———	———	———	———	———	———
Glycerine	0.03	———	———	———	———	———	———	———
Artificial flavor	0.30	———	———	1.02	1.12	———	1.01	1.18
Sugar	———	———	———	14.26	———	3.66	14.10	———
Water (variable)								

(1) Prepared by pressing.

(2) Soaked, peeled, cooked at atmospheric pressure, dried, milled.

The ingredients of these pellets are shown on Table 6. The proximal analysis and the PER of pellets 102 and 106 were the most tasty ones and are shown in Table 7.

The discovery of this new type of protein supplement opened new possibilities on the use of natural food such as nuts or different processed products such as pellets of protein concentrates made of cottonseed, cheese, Kamaboco (fish protein concentrate), candied fruit, raisin, etc. There was now a need to make comparisons and establish their relative importance as protein enrichers.

TABLE 7
PROXIMAL ANALYSIS OF TWO TYPES OF PROTEIN SHOTS MADE FROM PALLARES

	WHITE (102) %	BROWN (106) %
Moisture	14.26	13.13
Protein	18.96	18.83
Fat	10.49	10.17
Fiber	0.80	0.95
Ash	2.63	2.50
Nitrogen free extract	52.86	54.42
PER	1.73	

To answer these questions, nutritional tests were made with bread containing 5 and 10% of these supplements just adding them to the dough or mixing them with the dough. Table 8 shows how any of these enriching agents raise the protein content of the sweet potato bread. In the case of pallares protein pellets, the protein content goes above 10.40, to 13.20% when these were added at the 10% level.

The results of nutritional studies done in laboratory animals are shown in Table 9, parts I and II and in Graph 3. It may evident that when 5% sweetened pallares or 5% toasted peanuts or 5% Brazil nuts were added, the nutritional value of the "Camote Pan" went up moderately. The Protal (cottonseed flour) balls at 5% did little better as was the case with salty bean paste at the 10% level. Cheese and Kamaboco at 5% improved

the PER a little further to 0.84-0.87. But when pallares pellets formula 102 were used, or casein at 5% the results were notably better with the PER standing at 1.26 and 130 respectively. This is only surpassed by L. M. fish protein used at the 5% level.

The protein value of sweet potato bread is also improved when the sucrose content is reduced from 15% to 2% but unfortunately the acceptability goes down correspondingly.

In general terms from the point of view of acceptability the results can be considered rewarding and although in some cases the sweet potato bread has been acceptable, none have been as good as the original product.

The results of figure 10 show that none of the enriched breads have as high acceptability as the candied fruit with the taster's panel. A probable explanation is that the panel, as well as the public in general is now qualifying a specific type of sweet bread, and any variations from the original are penalised, even though in some cases the bread is of good flavour and appearance. For this reason it seems that it is sometimes easier to create a new product than to modify one already in existance when it has already been accepted by the public. In previous studies on "Camote Pan" similar drop in acceptability came about when, for economy's sake, the candied fruit was replaced by raisins. It took some time to reaccustom the public to the new flavour before the original high ratings were given again to the bread.

This reasoning could explain why the bread had such low acceptability when it was enriched with Brazil nuts, the toasted peanuts, the beans or pallares preparations. The fact that the bread enriched with cheese got high marks confirms the possibility of creating new products, as the cheese bread could not have been more different from the control bread made with candied fruit.

The inclusión of protein pellets in the sweet potato bread was not a success from the point of view of flavour. It lost aroma and texture during the production; for this reason we are still working on a protein pellet more suitable for the sweet bread. Meanwhile it has been found that the same pellets are attractive when eaten alone as a sweet or a snack. As it is a tasty product and entirely new it will probable be successful.

The concept of a protein pellet seems particularly indicated for the enrichment of bread because:

TABLE 8
PROTEIN AND FAT CONTENT OF SWEET POTATOE (CAMOTE-PAN) BREAD FLOURS (DRY BASIS)

	PROTEIN %	FAT %
1. Camote-Pan with candied fruit	10.49	10.50
2. " " beans (ph. lunatus) 5%	12.21	6.50
3. " " peanuts 5%	11.71	8.09
4. " " Brazil nuts 5%	12.99	10.11
5. " " Protal balls 5%	11.73	6.26
6. " " beans (ph. vulgaris) 10%	11.43	6.26
7. " " candied fruit 5% with 2.5% sugar	10.78	6.53
8. " " cheese 5%	12.14	6.86
9. " " Fish proteins commercial 5% (Kamaboco)	12.84	6.62
10. " " Casein 5%	17.44	4.80
11. " " Fish protein UI 5%	11.54	6.85
12. " " Protein shots from beans (ph. lunatus)	21.82	6.49

TABLE 9 (I)
PER OF SEVERAL ENRICHED SWEET POTATO BREADS (1)
Part. I

	Control Casein	CAMOTE-PAN (2) with				
		Candied fruit(3) 5%	Pallares(4) 5%	Peanuts(5) 5%	Brazil nuts(3) 5%	Protal Balls (6) 5%
. Initial weight, g.	43.85	40.74	44.80	43.13	44.25	43.35
. Final weight, g.	91.20	40.18	46.98	45.84	46.52	50.32
. Weight gain, g.	47.35	-0.57	2.18	2.71	2.27	4.97
. Feed consumption/ animal, g.	162.40	92.75	120.34	112.94	99.37	25.97
. % of protein in the diet	10.89	9.00	9.48	11.20	9.40	10.55
. PER	2.68	-0.10	0.19	0.21	0.24	0.37
. PER % of casein	100	———	7.09	7.84	8.96	13.81
. Duncan test 0.05						

) For enrichment the 5% candied fruit is substituted by various foods

) Formulae F-2 cake type where 12% wheat has been substituted by sweet potato pure, contains 15.4% sugar and 5% candied fruit.

) Inserted in the dough as such

) Inserted in the dough as a paste

) Inserted in the dough after toasting

) Inserted in the dough as such; formula: 5% cotton seed flour, 5% ground crackers, condensed milk, instant coffee, grated coconut, and licor (pisco).

TABLE 9 (II)
PER OF SEVERAL ENRICHED SWEET POTATO BREADS (1)
Part. II

	CAMOTE - PAN (2)						
	Cooked common Beans (3) 10%	Macerated cheese hard (4) 5%	Fish protein commercial(5) 5%	Pallares protein shot (102)(4) 10%	Casein (6) 5%	Fish protein L.M. (7) 5%	Candied fruit with 2.5% sugar in camote pan (8) 5%
1. Initial weight, g.	44.00	43.10	43.20	44.72	45.13	43.00	43.21
2. Final weight, g.	51.92	53.93	55.37	69.45	67.55	65.27	53.23
3. Weight gain	7.92	10.74	12.27	24.73	22.42	22.27	10.03
4. Feed consumption/ animal, g.	118.84	127.33	130.15	191.70	171.25	152.64	131.29
5. % of protein in the diet	10.37	10.10	10.73	10.27	10.00	10.37	10.10
6. PER	0.64	0.84	0.87	1.26	1.30	1.41	0.76
7. PER %	23.83	31.34	32.46	47.15	48.51	52.61	28.36
8. Duncan test (0.05)		———		———			

(1) For enrichment the 5% candied fruit is substituted by various foods.

(2) Cake type, where 12% wheat has been substituted by sweet potato puree, contains 15.4% sugar and 5% candied fruit.

(3) Inserted in the dough as a salty paste.

(4) Inserted in the dough as such.

(5) Commercial "Kamaboco". Inserted in small cubes.

(6) Mixed with the dough.

(7) Mixed with the dough. Prepared by the department of Nutricion UNA.

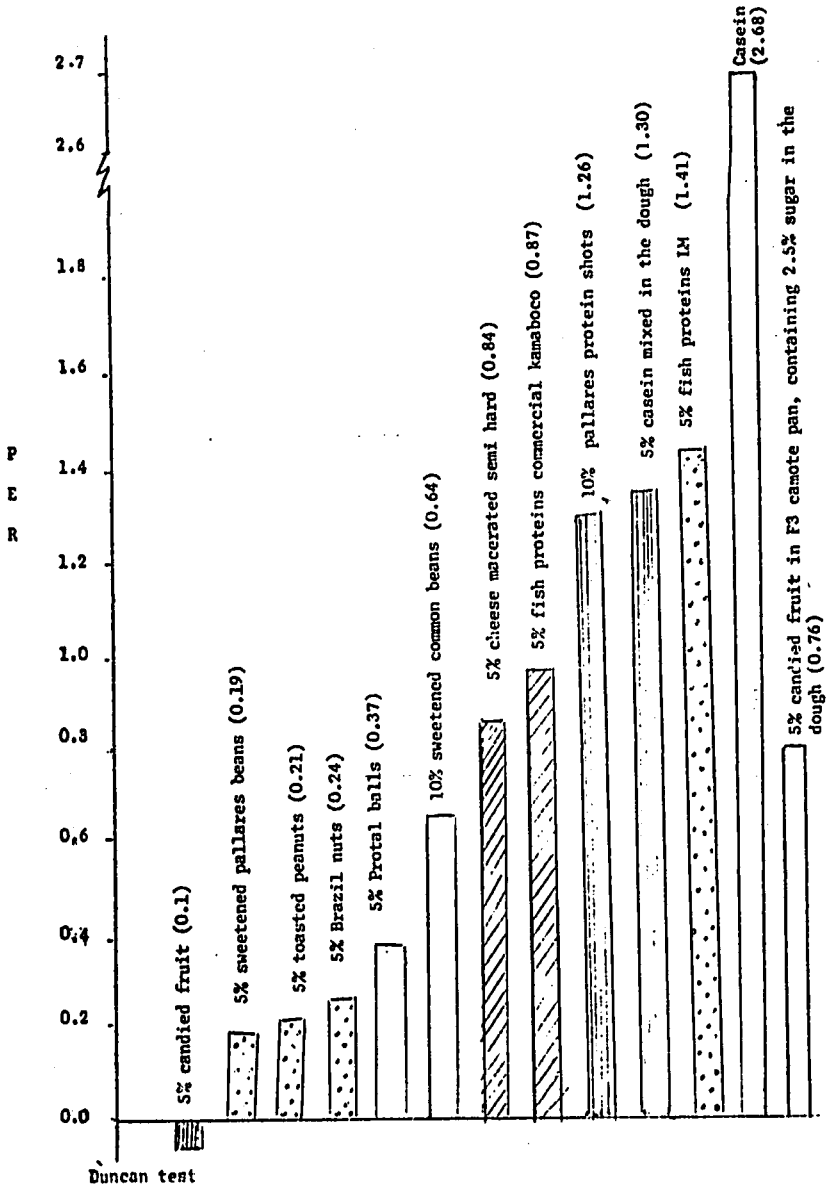
(8) Formula F-3.

TABLE 10
PANEL TESTING RESULTS FOR SOME ENRICHED BREADS (1)

	CAMOTE - PAN WITH							
	5% Candied fruit	5% Pallares (ph. lunatus) (2)	5% Peanuts	5% Brazil Nuts	5% Protal Balls (3)	10% Common Beans (ph. vulgaris)(2)	5% Cheese	Fish Protein L.M. (4)
TASTE								
Very good (5)	80	—	—	10	15	—	60	—
Good (4)	18	5	70	30	60	6	30	20
Fair (3)	2	70	20	40	20	71	35	70
Poor (2)	—	25	10	20	5	25	5	8
Very Poor (1)	—	—	—	—	—	—	—	2
FRAGANCE								
Very good (5)	25	5	—	—	22	6	20	—
Good (4)	70	10	60	40	40	9	40	20
Fair (3)	5	15	40	60	22	16	20	70
Strange (2)	—	70	—	—	16	60	20	8
Disagreable (1)	—	—	—	—	—	—	—	2
TEXTURE								
Very hard	—	—	—	—	—	—	—	—
Hard	—	—	50	40	10	—	10	—
Right	70	80	20	50	70	80	70	20
Soft	35	20	30	10	20	20	20	70
Very soft	—	—	—	—	—	—	—	10
Bread volume cc	427	420	448	410	445	416	484	430
Weight, g.	145	140	142	144	140	143	145	144

(1) Average of 20 tasters per sesion
(2) Cooked and strained beans
(3) Protal Balls 50% (cotton seed flour, 50% condensed milk, instant coffee, grated coconut, licor (pisco)
(4) Fish proteins LM - Prepared in the Department of Nutrition UNA.

GRAPH 3
ENRICHMENT OF CAMOTE PAN (F2) CONTAINING 15.4% SUGAR



1. It reduces the Maillard reaction in the baking of the ead, and the crumb keeps its normal colour, as opposed to eads made with wheat flour enriched with protein concentrates ch as fish, cottonseed, soya etc. which produce a dark crumb proportion to the concentration of protein concentrate used.

2. The pellet permits the use of a larger percentage of protein ithout altering the texture or colour of the crumb or crust.

3. It allows variations in the formulation of the pellet as flavour colour shape smell and texture.

4. It permits an increase in the nutritive value of the product it isolates the protein concentrate from elements in the crumb hich could react with it destroying or blocking the free amino ids and the proteins.

5. It permits the use of proteins of high nutritional quality hich do not have good functional properties for bread-making.

The are many protein concentrates which can easily be turned to pellets but there are relatively few proteins which can be cessfully incorporated into bread.

Finally it is possible that with the use of protein pellets many nown foods may be imitated, and new ones rich in proteins ay be made according to demand or necessity. For example otein pellets that are salty or sweet, which combine the attraction the salty or sweet outer layer with the nutritional value of e cooked and flavoured beans in the centre.

It is an intriguing idea to use this type protein of pellet for ildren and adults as it could offer a valuable alternative to e empty calories of most present day sweets and snack foods.

SUMMARY

Phaseoulus vulgaris and Phaseoulus lunatus have been used for protein richment of pasta. The addition of cooked beans, equivalent to 15-40% wheat ur replacement, have not shown major technological difficulties and have reased the nutritional value of the noodles considerably. The same beans have en used for protein enrichment of a sweet bread: "camote pan", containing eet potatoes (Hipomea batata), wheat flour, sugar and lard. The cooked ans were added either as a paste or as protein pellets. The PER value of "camote pan" was raised from a negative value to 0.28 and 0.32 when and 10% of a bean sweetened pasta (Phaseoulus lunatus) was mixed with dough. This value went up to 0.84 when it was inserted, without mixing th the dough, in order to minimize reactions of proteins and sugars. When otein pellets, containing no sugar were inserted in the dough the PER went to 1.26, which demostrates the extensive damage that the Maillard reaction erts on the nutritional value of the sweet bread studied. Protein pellets made m beans have very good possibilities as candies or snack foods due to ir high nutritional value and excellent acceptability.

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Toxic factors in beans. Their practical importance

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Common or garden beans *(Phaseolus vulgaris)* are important in the staple diet of most American nations and also in some parts of Africa and India (1). Black beans dating back to several centuries B. C. and similar to those still consumed today, have been found in indian tombs of Perú. How can toxic factors exist in such widly used food? This apparently contradictory fact can be explained because most of the toxic and antinutritional factors in beans are destroyed by proper cooking and the remaining thermostable factors or properties are not sufficiently harmful to outweigh the positive nutritional qualities of beans. Nevertheless, a thorough knowledge of the toxic factors, both thermostable and thermolabile is of great importance for avoiding, as far as possible, any negative influence under any mode of consumption and to enable food technologists and plant breaders to reduce or eliminate these factors in their respective products.

There are four more or less well defined thermolabile toxic factors, the biochemical activities of which are fairly well known. These are three enzyme inhibitors and the toxic hemagglutinins. The relation of these factors to possible toxic effects of crude or poorly cooked beans will be discussed.

Other properties of beans which limit their nutritional usefullnes are: poor cooking quality, poor digestibility, low level of sulfur amino acids, and flatulence production. It is not always easy to distinguish between these different factors in regard to their activity upon growth performance of experimental animals.

TABLE 1
DIGESTIBILITY OF RAW AND COOKED LEGUMES

SAMPLE	No. OF SAMPLE	RAW	COOKED	TRYPSIN INHIBITOR U/g
RED BEANS	4	52.4	77.9	13.2
BLACK BEANS	4	51.3	79.2	51.5
MOTTLED BEANS	4	52.8	83.5	28.1
WHITE BEANS	4	53.3	89.3	20.4
PEAS	4	91.5	91.5	0
LENTILS	4	89.7	87.2	0
CHICK PEAS	4	90.7	88.8	15.7
COW PEAS	4	79.9	86.7	21.7
CONGO PEAS	2	78.7	84.1	25.9

Heat-labile factors would be of no importance if their destruction could be achieved under all cooking conditions. Primitive cooking is often done in earthern pots on wood fire. With a rough, viscous mass like cooked beans, heat transfer in necessarily imperfect and in the absence of constant and vigoreous stirring the temperature reached in part of the preparation may be well below the boiling point. Another factor to be reckoned with is the low boiling point of water at high altitudes. Therefore, the complete destruction of thermolabile factors may not always be achieved. Conclusions from laboratory experiments on cooking under controlled conditions, often in the autoclave, cannot always be applied to practical field conditions. In the following study we have included cooking at different temperatures for 2 h in order to investigate the destruction of heat labil factors under unfavorable conditions.

Trypsin inhibitors exist in many legumes. They have been studied extensively both in their chemical and biological aspects (2). Their toxicity is low and no direct relation to digestibility is apparent (Table 1). Moreover, they are destroyed more readily than the other heat-labile factors (Table 2). There are bean cultivars free of trypsin inhibitor (Table 3). Nothing is known, however, about the inheritance of these factors. It seems doubtful

whether attemps to produce bean lines free of these factors by genetic selection would be a worthwhile undertaking. The isolated inhibitors contain 6-12 cystine residues (3). High trypsin inhibitor content may be related to high sulfur amino acid levels in beans and therefore may be desirable. The data from Table 3 show that some bean seed extracts also inhibit chymotrypsin while others don't. This can be explained only by the existence of more than one class of inhibitory factors. If both enzymes were inhibited by a single multispecific inhibitor, the relation between the activies toward the two enzymes should be constant. Indeed, several different inhibitors have been isolated from beans (3).

TABLE 2

INFLUENCE OF HEAT TREATMENT ON HEMAGGLUTININ, ENZYME INHIBITORS AND DIGESTIBILITY OF RED BEANS

	Trypsin Inh. U/g	Amylase Inh U/g	Hemaggl.	Digestibility
Raw	37.7	144	11	30.0
Cooked at 98°	0	0.3	0	82.1
Cooked at 90°	0	17.0	2	64.8
id. with acetic acid	0	11.4	2	59.4
id. with $NaHCO_3$	0	8.0	2	66.2

A non-specific proteinase inhibitor can be separated from the specific trypsin and chymotryprin inhibitors in beans extracts (4). In Fig. 1 the results of an experiment are presented, which show that stepwise adsorption of a bean extract with bentonite-celite will remove 90% of the trypsin inhibitor activity in 6-8 steps. The remaining 10% of activity is not affected by further treatment, although the protein content of the extract is still declining. This result proves that besides the specific trypsin inhibitor there exists in the bean extracts another factor which acts not only on trypsin but on all of the proteinases studied (Table 4).

TABLE 3

ENZYME INHIBITORS AND DIGESTIBILITY OF SELECTED BEAN SAMPLES

No.	SAMPLE	TRYPSIN INH. U/g	QUIMOTRYPSIN INH. U/g	DIGESTIBILITY CRUDE	DIGESTIBILITY COOKED
2035	RED BEANS	0	0	49	71
2311	" "	39	22	56	83
2081	BLACK BEANS	65	17	55	80
2041	WHITE BEANS	0	11	52	91

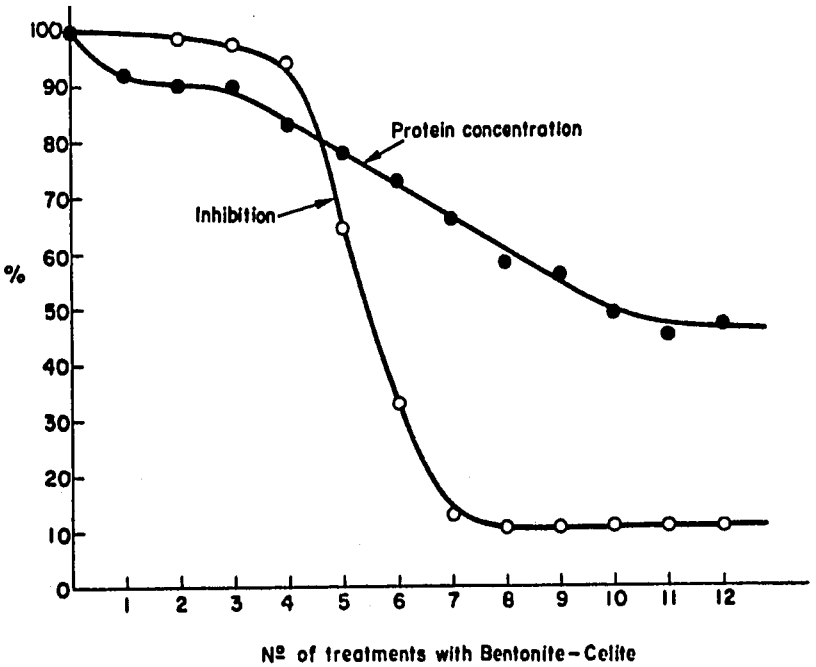

Figure 1
Protein concentration and trypsin inhibitor activity in bean extracts after repeated treatments with Bentonite-Celite.

TABLE 4
ENZYME INHIBITION BY BEAN GLOBULIN-E MEASURED WITH DIFFERENT METHODS

Substrate	Enzyme	Method	Globulin E added mg	Activity %
Casein	Trypsin	Absorption at 280 mu	4	65
Casein	Trypsin	Formol titration	4	57
TSAA	Trypsin	NH_3 microdiffusion	4	65
Egg albumin	Papain	Absorption at 280 mu	4	68
Casein	Papain	Absorption at 280 mu	4	63

This inhibitor is identical or closely related to one of the globulin fractions of beans (4). Its activity is not completely abolished even after boiling, and it may therefore be related to the rather poor digestibility of cooked beans (5). The inspecific inhibitor is much less active on a weight basis than the trypsin inhibitors. Indeed, the trypsin inhibitor test does not easily detect the inspecific inhibitor because it is performed with an insufficient amount of bean extract. But the level of the globulin fraction, which may be identical with this inhibitor, is rather high in bean seeds and for this reason it may have some practical importance on the digestibility of beans. Its more detailed study would be of considerable interest.

Amylase inhibitors exist in many legume seeds (6). Among 42 samples of beans we found only one free of this inhibitor (Table 5). Its action is reduced by cooking, but some activity can be detected even in well cooked beans (Table 2). All bean seeds exhibit some amylase activity which is not affected by the bean amylase inhibitor, which acts only on pancreatic and salivary amylase (6). The fact that undigested starch may appear in considerable concentration in feces of rats fed diets prepared with beans rich in amylase inhibitor (5) indicates that it may have some practical importance. The use of crude legume seeds containing amylase inhibitor for the preparation of animal feed mixes should be viewed with caution. It seems important to study the relation between amylase inhibitor and metabolizable

energy in animal feed. Further experiments should clarify whether the small residual activity in beans which resists cooking (Table 2) has any nutritional significance for humans.

TABLE 5
AMYLASE INHIBITOR ACTIVITY IN ETRACTS FROM SEEDS OF DIFFERENT LEGUMES

Common name	Scientific name	Number of samples tested	Range of inhibitor activity Units/g
Kidney beans	Phaseolus vulgaris	42	40 - 268 1/
Lima beans	Ph. lunatus	14	33 - 120 1/
Runner beans	Ph. coccineus	9	73 - 150 1/
Wild beans	Ph. aborigineus	6	108 - 132
Adzuki beans	Ph. angularis	2	64 - 92
	Ph. acrifolius	1	62
	Ph. atropurpureus	1	60
	Ph. trilobus	1	52
	Ph, acutifolius	4	51 2/
	Ph. semierectus	1	60
	Ph. geophilus	1	70
Mung beans	Ph. aureus	1	32

1/ One sample without detectable inhibitor activity

2/ Three samples without detectable inhibitor activity

There is a direct relation between heat treatment and digestibility. The conditions for autoclaving, heating of the water soaked beans to 118° for 20 min., were chosen so as to result in optimal digestibility (Fig. 2). This figure shows the significant influence of the heating-time on digestibility (13). It must be pointed out that the three lots of beans used for this experiment had been stored for about 8 months without special precautions. This fact may have influenced the outcome of the cooking trial which probably depends on the freshness of the beans.

The bean seed coats are less easily digested than the whole seeds. Dehulling or straining of beans increases digestibility (Table 6). Therefore, strained beans are recommended for use in child feeding.

Progress has been achieved lately in the evaluation of the nutritional importance of bean hemagglutinins. Four different types of agglutinins have been defined by their respective activities

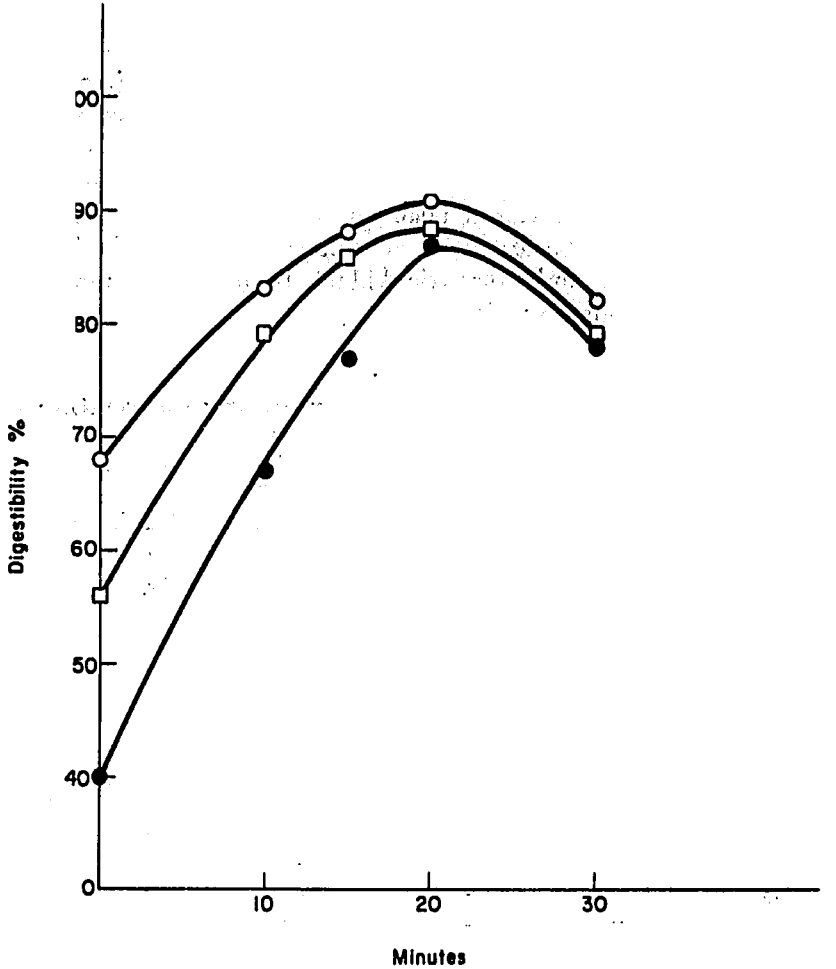

Figure 2

Digestibility of 3 different bean samples after 2 h soaking in water and subsequent autoclaving at 15 lb pressure for different lengths of time.

TABLE 6

DIGESTIBILITY OF COOKED DEHULLED BEANS AND SEED COATS

	Endosperm	Coats
Black beans	79.2	36.7
Red beans	81.2	40.3
Mottled beans	86.5	45.4
White beans	87.4	71.8

when tested with different blood preparations (7). Using rabbit blood and trypsin-treated cow blood cells the four agglutinin types which have been named A, B, C and D, can be distinguished (Table 7). Only two of these have significant toxic activity as shown in the dose-mortality curves of mice injected with different amounts on the extracts from the four bean types (Table 7) (8). It must be emphazised that the test with cow blood cells which distinguishes between toxic and non-toxic kidney bean types, cannot be used for other legume species.

TABLE 7
HEMAGGLUTINATING SPECIFITY AND TOXICITY OF EXTRACTS OF 20 BEAN CULTIVARS

Cultivar [1]	Agglutination with		Bean type [2]	Toxicity [3]
	Rabbit RBC	Trypsin treated cow RBC		
Balin de Albenga	+	+	A	5/4
Mérida	+	+	A	9/9
Negro Nicoya	+	+	A	5/4
Saxa	+	+	A	5/5
No. 755	+	+	A	5/5
No. 756	+	+	A	5/5
Peruvita	+	-	B	5/0
Palleritos	+	-	B	6/0
Juli	+	-	B	5/0
Cubagua A	+	-	B	5/0
Porillo	-	+	C	5/5
Negra No. 584	-	+	C	5/3
Vainica Saavegra	-	+	C	10/6
Rabuda	-	+	C	5/5
Hallado	-	-	D	5/0
Madrileño	-	-	D	5/0
Alabaster	-	-	D	5/0
Triguito	-	-	D	6/0
Mountaineer Half Runner	-	-	D	8/0
Great Northern 1044	-	-	D	5/0

1 Extracts prepared with 1 g of ground bean seeds and 5 ml of pypiological saline; for hemagglutinin tests diluted 1:10.

2 Classification according to the hemagglutinating specifity.

3 Mice were injected with a single dose of 1 ml of undiluted bean extract/20 g of body weight by the intraperitoneal route, injected mice/mice dead within 3 days.

The difference between bean cultivars belonging to one of the "high-toxic" or to one of the "low-toxic" types can also be demostrated by feeding experimental diets containing one of these ground bean types supplemented with methionine and a casein digest (5). The data of Table 8 show that rats ingesting the toxic raw bean diet will lose weight and die within 10 days, while those receiving the diet prepared with low-toxic beans grow satisfactorily. Heating in water to 90° for 2 h will not detoxify completely the red beans. Hemagglutinating action on trypsin-activated cow red blood cells is still detectable under these experimental conditions (8) (Table 2).

TABLE 8
PERFORMANCE OF RATS FED DIETS PREPARED WITH 40% OF GROUND BEANS AND 0.3% OF DL-METHIONINE

Diet	Bean used : Type A (red)	Type D (white)
	Wt change/day g	
Raw beans	- 2.1 *	+ 0.1
Raw beans + 10% digested casein (Casitone)	- 1.8 *	+ 2.3
Beans cooked at 85° for 2 h	- 0.5	+ 1.5
Beans autoclaved at 118° for 30 min	+ 2.8	+ 2.4
Beans autoclaved without methionine	+ 0.4	+ 0.3

* All animals died within 10 days

When single seeds of commercial bean cultivars are tested for hemmaglutinin specifity the presence of seeds of two and sometimes three bean types can often be detected. The inheritance of the toxic A-type hemagglutinin was tested by crossing A and D-type plants (7). In the F_2 generation, 75% of A-type and 25% of D-type plants were observed pointing to the fact that the A-type character is inherited in this case as a single dominant trait (Table 9). It should therefore be easy to obtain pure type D-line beans of low toxicity by harvesting the seeds from single plants and determining their type by the specific blood agglutinating test. Indeed, from a Venezuelan commercial black bean cultivar, which proved to contain type A and B seeds, we have obtained

a pure B-type line of black beans by selecting the seeds from B-type plants.

TABLE 9

HEMAGGLUTINATING ACTIVITY IN THE F_2-GENERATION OF CROSSES OF TYPE A AND D BEAN PLANTS

No.	Type of cross	Agglutination of rabbit and trypsin-treated cow rbc Number of seeds +	-
1	A/D	23	13
2	A/D	76	28
3	A/D	22	14
4	D/A	30	6
5	D/A	55	22
6.	D/A	241	81
7	D/A	231	77
		678 (73.7%)	241 (26.3%)

Extracts from each seed were tested with rabbit and trypsin-treated cow blood cells separately. All positive seeds showed activity with both blood types.

Mixtures of ground beans and ground cereals have been used in child feeding programs (9). These mixtures can be prepared locally from easily available materials and can be formulated in a way that the proportion of legume and cereal proteins results in optimal supplementation. When cooked, these mixtures will require only a short heating time to become palatable. The hemagglutinins are not always destroyed under these conditions (10). It should been born in mind that food with a low content of toxic hemagglutinins may not produce the symptoms known to occur from bean intoxication (11) but may reduce the intestinal absorption and in this way the food value (12). This point needs further clarification. In any case, it seems desirable that for all uses of ground beans cultivars of low toxicity are selected.

Beans which due to some storage damage are unsuited for human consumption have been used occasionally for animal feeding with rather poor results. A better understanding of the differences in the degree of toxicity between different bean cultivars will be helpful in avoiding these unfavorable experiences.

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SUMMARY

Most proteinase inhibitors in beans are thermolabile and are destroyed by proper cooking. Some bean cultivars do not contain measurable amounts of trypsin-or chymotrypsin inhibitor activity. An unspecific proteinase inhibitor found in the globulin-fraction of bean proteins was rather thermostable. Amylase inhibitor activity is found in most but not all bean cultivars. In beans cooked at 90° some of this activity is still detectable.

Four different types of hemagglutinins can be distinguished in different bean cultivars. They differ in toxicity, heat resistance and specificity toward different blood preparations. The most toxic type is inherited as a single dominant factor. In food preparations of mixtures of ground beans and cereals the thermolabile factors are not always completely destroyed by cooking.

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Problems in the exploitation of natural variation in composition of legumes

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INTRODUCTION

Food scientists and nutritionists have tended to place individual food crops into certain groupings and make generalizations about the composition of the foods in these groups. This generally has proved to be a valid procedure and it has been found that diets are often established on the basis of appropriate combinations from different groups, the nutritional deficiencies of one being offset by the sufficiencies of another. This balancing role is ideally performed using the foods indigenous to a population. The legumes offer several advantages in a mixed diet (2, 7) because of their high proteing levels produced at a low cost. In addition, they may contribute significantly to other human dietary requirements (5). Their low levels of sulfur-bearing amino acids have generally limited their contribution in a mixed diet. Increases of methionine and/or cystine ranging from 300% in *Phaseolus vulgaris* to 50% in *Glycine max* would bring the sulfur amino acid level up to that of egg, and would establish tryptophan as the limiting amino acid on the way up. Then, it would be desirable to increase tryptophan. The most permanent and least costly (in the long run) way to change plant composition is through plant breeding. The practicality of this approach with corn has been demonstrated since the discovery of the *opaque*-2 mutant (8). A similar breakthrough has been reported very recently in sorghum (10).

One cannot expect that such dramatic genetic discoveries involving single recessive alleles will take place with all grains. It is more likey that most crop breeders will be content with a series of small qualitative or quantitative protein increases. The significance of such small improvements cannot, however, be underestimated when measured over vast acreages for many years.

Variability is the most important resource at the hands of a plant breeder. It provides him with the genes for disease resistance, color, flavor, plant type, seed size and shape, maturity date, yield, etc. At the same time, this variability presents him with a difficult problem of managing huge populations. The more characters he tries to combine, the greater his populations must be if his likelihood of recovering the best combinations is to be high.

Adams (1) illustrated that the 99% assurance of recovery for five independent recessive gene pairs for high protein in a backcross program would require a population in excess of 150,000 plants. Even for an easily observed character such as seed type, this presents no small task. But, even after selecting out the readily observed traits, one is left with a very difficult analytical problem of selecting the desired protein types. With the occurrence of dominant genes for the desired type, the populations are reduced. And with the occurrance of multigenic inheritance, the breeder cannot predict accurately what populations he will need. He must then work with whatever numbers he can reasonably handle. This obviates the need for simple, rapid and inexpensive screening techniques.

Stevens (11) pointed out the problems a plant breeder encounters when trying to determine which characters he is to incorporate into new varieties. He stated that it is simply not possible to develop a cultivar with all the desired characteristics. The number of phenotypes possible in an F_2 generation from parents differing in 21 allelic pairs exceeds ten billion. With a plant population of 500,000/ha, over 20,000 hectareas would be required to observe this population. And even at that perfect population, a lot of luck and keen observational skill would be required to recover the ideal progeny.

In actual practice, plant breeders do not deal with such large populations because they are able to use breeding and selection techniques which significantly increase their efficiency, including mass selection, seedling screening, marker genes *et al.* Nonetheless, obtaining large populations and using rapid selection methods is the key to success.

Legume breeders have not yet made significant progress toward the quantitative or qualitative protein improvement in varieties. Johnson and Lay (3) gave an up-to-date summary of progress in the genetic improvement of protein in cereals, legumes and potatoes,

and their summary of efforts in this direction certainly indicates that there is great potential in further research on protein improvement by genetic means.

The Protein Advisory Group of the United Nations, following an international Symposium and Workshop involving legume breeders and supporting scientists in 1972, published a definitive statement (9) outlining a plan of action for the upgrading of human nutrition through the improvement of legumes. Genetic improvement would be a major part of this program, but an extensive interdisciplinary approach is strongly supported.

The breeder, always mindful of the problem mentioned above, can contribute to the nutritional improvement in any or all of the following areas - provided his program is granted a sufficient priority by his funding agency.

Increased yield, perhaps the most difficult to define genetically, can be one of the most important means of feeding more people. The yield problem is particularly important in legumes where there have been few major advances. This problem involves photosynthetic efficiency, disease resistance, environmental adaptability and a host of poorly defined characters. We do not know what our top yields might be for most legumes.

Increased total protein content, almost without regard to protein quality, but not without regard to yield, offers the next best means of improving the nutritional contribution of legumes. Because legumes are likely to be consumed in a mixed diet, they can be important protein sources when consumed in combination with other foods higher in sulfur-bearing amino acids. We know that protein levels range from 17-35% in the common bean and from 31-52% in the soybean (6).

Improved protein quality, especially of methionine and cystine deficiencies, can probably be attained. Any improvements in this character would result in a reduced need for the total amount of legume or legume protein required and would thus be tantamount to a yield or protein increase. Work at the Campbell Institute for Agricultural Research (4) indicates that real genetic differences in at least one form of methionine, as measured microbiologically, do exist and might be exploited.

Protein digestibility and utilization must be determined at some stage in a breeding program if any quantitative protein and amino acid differences are to be meaningful. This work by nutritionists and biochemists can begin even before the breeder has made quan-

titative improvements, to be plugged into the breeding program at some later time.

Acceptability factors must be considered because no matter how good (nutritionally) we make a legume, it makes no contribution if it is not consumed. Thus, we add to the breeder the problems of inhibitors, color, size, shape, flavor, flatulence, texture and cooking quality.

Bonuses, such as increased levels of other essential amino acids, vitamins or minerals, should not be overlooked, especially if rapid, inexpensive selection methods are available.

The "numbers problem" of the plant breeder dictates that biochemists and nutritionists present him with the tools for selecting efficiently those genotypes which are actually better from the standpoint of human nutrition. It would be extremely wasteful for breeders to spend years developing a variety which is good "on paper" but of no special value in human diets. But, it would also be wasteful of time for the breeder not to proceed while he waited for a final answer from his supporting coworkers. It is proposed that we establish a comprehensive network of researchers working simultaneously in all related disciplines to increase the contributions of legumes in feeding hungry people. The programs would adjust their goals and procedures depending on the contributions from parallel programs; this would require excellent communication among workers in the various parts of the overall network. An extension program to educate people in the use of legumes as a source of protein will also be needed. Such a task force requires establishment of new priorities (spelled p-o-l-i-t-i-c-s) by governments and international agencies.

SUMMARY

The legume plant breeder has available to him different levels of variation in seed yield, protein level and amino acid composition, depending on species. There is a tendency among scientists in other disciplines to ascribe extraordinary powers to the breeder and to expect him to put together all the desirable characters which they feel are needed. The problems encountered in trying to put all these characters together make it necessary for the breeder to handle large populations, necessitating further the involvement of a multi-disciplinary attack upon the various facets of legume improvement programs.

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Amino acid supplementation of common beans and other legumes

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I. *Importance of Legumes*

Much attention has been devoted in the past several years to the role that increased utilization of legumes might play in alleviating problems of protein-calorie malnutrition in the less developed countries. One indication of this renewed interest in legumes is the fact that a recent issue of the PAG Bulletin is devoted almost in its entirety to this subject (1). The PAG Statement No. 22 includes the following comment, "The food legumes are important and economical sources of protein and calories as well as of certain vitamins and minerals essential in human nutrition. However the significant role they play in the diets of many developing countries appears to be limited by their scarcity, caused in great part by their present low yields, their consequent cost, and certain defects in their nutritional and food-use qualities" (2). Realization of the potential of legumes is not new, however, and thoughts similar to the above have been expressed over ten years ago (3, 4).

It is the purpose of this paper to examine one mechanism of the nutritional improvement of legumes, namely that resulting from amino acid supplementation, and to discuss practical aspects related to such supplementation. The review will consider common food legumes, such as peas and beans, that are consumed directly by human beings, and will not consider the oilseeds such as soy and peanuts. For a summary of studies on the amino acid supplementation of protein concentrates from these sources the reader is referred elsewhere (5).

II. *Amino Acid Composition and Nutritive Value*

It is well known that the first limiting amino acid in legumes is methionine, as contrasted with cereals where lysine is first limiting (6). It is not entirely an accident that much of mankind evolved over many centuries on diets consisting of mixtures of cereals and legumes. In Table 1 data on the amino acid composition and protein nutritive value of eight important legumes are given. It is apparent that all are good sources of lysine and relatively poor sources of the sulfur amino acids. In addition, pigeon peas, *Cajanus cajan,* are also seriously lacking in tryptophan. For these major legumes biological values ranged from 45 to 70 and digestibility from 70-90%. These averages can be misleading, however, and the digestibility of some varieties of beans is as low as 50-60%. Thus NPU's can be even lower than the values of 30-50 that are listed in Table 1. These indicators of protein nutritive value listed in Table 1 are based on studies using cooked legumes. It is well recognized that most all legumes have to be heat treated to destroy various toxic substances such as trypsin inhibitors and hemagglutinins (1).

TABLE 1

AMINO ACID COMPOSITION AND NUTRITIVE VALUE OF VARIOUS LEGUMES[1]

	mg/g N							
Legume	Lysine	Sulfur Amino Acids[2]	Threonine	Tryptophan	Chemical Score[3]	Biol. Value	Digestability	NPU
Phaseolus vulgaris	450	119	248	63	47	58	73	38
Phaseolus aureus	504	77	209	50	32	70	81	—
Phaseolus lunatus	465	141	261	50	50	66	78	52
Pisum sativum	470	127	254	56	50	64	88	47
Lens esculenta	449	107	248	60	41	45	85	30
Vigna sinensis	427	141	225	66	57	57	79	45
Cicer ariotinum	428	139	235	54	53	68	86	—
Cajanus cajan	481	93	182	35	39	57	78	52
Reference Standard								
Whole Egg	436	362	320	93	100	94	97	94

1. Data obtained from reference (5).

2. Methionine + cystine.

3. CS = $\frac{\text{limiting amino acid (mg/g essential amino acid nitrogen) in sample}}{\text{concentration of same amino acid (mg/g essential amino acid nitrogen) in egg}} \times 100$

III. *Amino Acid Supplementation*

The relative deficiency of sulfur amino acids in most legumes naturally suggests that their biological value can be improved by

supplementation with methionine. This has been amply demostrated to be the case as will be documented. The studies to be reviewed all deal with the effects of methionine addition on heat treated samples. The beneficial effects of methionine addition on raw legumes will not be considered.

The results of rat growth studies on the effects of amino acid supplementation of various legumes are summarized in Table 2. Studies have been reported using pinto beans (7), navy beans (8), black beans (9), and kidney beans (10). All these varieties of *Phaseolus vulgaris* responded markedly to methionine addition, as measured by rat growth. In general the effect was to convert a poor protein sources into one that supported adequate growth of the weanling rat. In two studies protein efficiency ratios (PER's) were calculated and as expected from the growth data the improvement in PER was substantiated in both cases.

In other studies mung beans (10), adzuki beans (10), lima beans (11, 7), and hyacinth beans (10) all responded to methionine addition by a considerable increase in weight gain. Among these varieties growth was best on supplemented hyacinth beans (4.4 grams/day) and poorest on one sample of lima beans supplemented with 0.1% DL-methionine (1.4 grams/day). However growth on another sample of lima beans supplemented with 0.2% DL-methionine was quite good (2.7 grams/day) suggesting perhaps that not enough methionine had been added in the first sample. It is also possible that varietal differences or differences in the effectiveness of heat treatment are involved since growth on the unsupplemented lima beans was much better with the second sample than with the first. The level of DL-methionine addition was 0.3% for all beans except the limas.

Studies on peas *(Pisum sativum)* also show a considerable increase in weight gain resulting from DL-methionine supplementation (7, 10, 11, 12, 13). Levels of addition ranged from 0.1% to 0.6% with no clear cut relationship between response and level. In general growth on the supplemented peas was not quite as good as growth on the supplemented beans. However, so many factors are involved such as varietal differences, effectiveness of heat treatment, growing conditions for the crop such as fertilizer level, assay conditions, and rat strain that it is not possible to conclude that supplemented beans do in fact contain protein that is more utilizable.

Rat growth studies with cowpeas, chickpeas, and pigeon peas are also summarized in Table 2. Methionine addition to chickpeas

more than doubled the weight gain from 1.2 to 2.7 grams/day. Growth on unsupplemented cowpeas, 2.2 grams/day, was better than on any of the other legumes. Methionine addition increased the daily weight gain to 2.9 grams.

As would be expected from the chemical score (Table 1) pigeon peas, or redgram, required addition of methionine and tryptophan in order to produce good growth. In one study methionine and tryptophan addition to redgram increased the daily weight gain from 1.2 grams to 3.6 grams. In the other study with pigeon peas daily weight gain increased from 0.2 grams to 3.1 grams as a result of supplementation with methionine and tryptophan. In summary all the legumes studied, when supplemented with methionine or in one case methionine and tryptophan, were able to support very adequate rat growth when fed at 10% protein in the diet. In view of the less intense need of the human for essential amino acids the results are suggestive that amino acid supplemented legumes would be an adequate protein sources for humans, especially since they generally contain over 20% protein. However because of the special problems connected with legumes, such as poor digestability and presence of toxic factors, it is important that the animal results be confirmed with controlled studies carried out with human infants.

IV. *Studies in Humans*

Studies in which the effect of adding methionine to beans or other common legumes on growth and/or nitrogen balance in human subjects have apparently not been reported. However, such data are available for methionine supplementation of soy protein, and these will be reviewed next.

Parthasarathy et al. (17) studied the effect on protein utilization of adding DL-methionine or DL-methionine hydroxy analog to soy flour fed to 8-9 year old children. The protein intake was 1.2 gram/kg and the supplmentation level was 1.2 grams/16 grams nitrogen. The results are summarized in Table 3. Methionine addition increased the biological value (BV) and net protein utilization (NPU) from 63.5 and 53.3 to 74.9 and 64.7 respectively compared to corresponding values of 82.6 and 72.0 for skim milk. The hydroxyanalog was nearly as effective as methionine itself. Korsland et al. (18) compared the effects on nitrogen balance of 12-16 year old boys of a textured soy protein (TVP) and a similar product

TABLE 2
AMINO ACID SUPPLEMENTATION OF COOKED LEGUMES. RAT STUDIES

Legume	Scientific Name	Protein Level %	Wt. Gain g/day	PER[1]	Reference
Pinto bean	Phaseolus vulgaris	10	0.3		(7)
Pinto bean + 0.3% DL-Methionine		10	1.9		
Navy bean	Phaseolus vulgaris	10	1.5	1.9	(8)
Navy bean + DL-Methionine[2]		10	3.4	3.1	
Black bean	Phaseolus vulgaris	12	1.9	1.2	(9)
Black bean + 0.3% DL-Methionine		13	4.5	2.4	
Kidney bean	Phaseolus vulgaris	10	0.0		(10)
Kidney bean + 0.3% DL-Methionine		10	2.8		
Mung bean	Phaseolus aureus	10	0.5		(10)
Mung bean + 0.3% DL-Methionine		10	2.3		
Adzuki bean	Phaseolus angularis	10	1.0		(10)
Adzuki bean + 0.3% DL-Methionine		10	1.8		
Lima bean	Phaseolus lunatus	10	-0.1	-0.2	(11)
Lima bean + 0.1% DL-Methionine		10	1.4	1.7	
Lima bean	Phaseolus lunatus	10	0.9		(7)
Lima bean + 0.2% DL-Methionine		10	2.7		
Hyacinth bean	Dolichos lablab	10	-0.3		(10)
Hyacinth bean + 0.3% DL-Methionine		10	4.4		

TABLE 2 (Continued)
Page No. 2

Legume	Scientific Name	Protein Level %	Wt. Gain g/day	PER[1]	Reference
Green pea	Pisum sativum	10	0.3	0.5	(11)
Green pea + 0.1% DL-Methionine		10	1.7	2.0	
Southern pea	Pisum sativum	10	0.7		(7)
Southern pea + 0.2% DL-Methionine		10	1.7		
Green pea	Pisum sativum	10	0.0		(10)
Green pea + 0.3% DL-Methionine		10	1.2		
Field pea	Pisum sativum	10	0.6	1.0	(12)
Field pea + 0.3% DL-Methionine		10	1.9	2.0	
Field pea	Pisum sativum	10	0.3	0.6	(13)
Field pea + 0.6% DL-Methionine		10	2.1	2.4	
Lentils	Lens esculenta	10	-0.1		(10)
Lentils + 0.3% DL-Methionine		10	0.5		
Lentils	Lens esculenta	10		0.9	(14)
Lentils + 0.6% DL-Methionine		10		2.5	
Cowpea	Vigna sinensis	12	2.2		(15)
Cowpea + 0.3% DL-Methionine		12	2.9		
Chickpea	Cicer aristinum	10	1.2	1.8	(11)
Chickpea + 0.1% DL-Methionine		10	2.5	2.7	
Chickpea	Cicer aristinum	10	1.3		(10)
Chickpea + 0.3% DL-Methionine		10	2.9		

TABLE 2 (Continued)
Page No. 3

Legume	Scientific Name	Protein Level %	Wt. Gain g/day	PER[1]	Reference
Redgram	Cajanus cajan	10	1.2	1.8	(16)
Redgram + DL-Methionine + L-Tryptophan[3]		10	3.6	3.2	
Pigeon pea	Cajanus indicus	10	0.2		(10)
Pigeon pea + 0.3% DL-Methionine + 0.08% L-Tryptophan		10	3.1		

1. Protein Efficiency Ratio = grams weight gained/grams protein consumed.

2. Methionine added up to level of 1957 FAO Pattern (4.2 grams/100 grams protein).

3. Methionine and tryptophan added at levels of 2.8 and 1.5 grams/16 grams nitrogen respectively.

supplemented with 1% DL-methionine. At a nitrogen intake of 5.0 grams/day methionine addition increased nitrogen balance from - 0.08 to 0.48 grams/day (Table 3). Kies and Fox (19) carried out a similar study with TVP at two levels of nitrogen intake in adult men. The results are also summarized in Table 3. At a nitrogen intake of 4.8 grams/day, methionine increased nitrogen balance from 0.70 to 0.45 grams/day. At a nitrogen intake of 8.8 grams/day, methionine addition had no effect on nitrogen balance.

TABLE 3
METHIONINE SUPPLEMENTATION OF SOY PROTEIN STUDIES IN CHILDREN AND ADULTS

Protein Source	Age of Subjects Years	Protein Intake g/Kg/day	BV[1]	NPU[2]	Reference
Soy flour	8-9	1.2	63.5	53.3	(17)
Soy flour + DL-Methionine hydroxy analog[3]			71.5	61.4	
Soy flour + DL-Methionine[3]			74.9	64.7	
Skim milk			82.6	72.0	

Protein Source	Age of Subjects Years	N Intake g/day	N Balance g/day	Reference
Textured Vegetable Protein	12-16	5.0	-0.08	(18)
Textured Vegetable Protein + 1% DL-Methionine			0.48	
Beef			0.32	
Textured Vegetable Protein	"Adults"	4.8	-0.70	(19)
Textured Vegetable Protein + 1% DL-Methionine			-0.45	
Beef			-0.30	
Textured Vegetable Protein	"Adults"	8.8	0.78	(19)
Textured Vegetable Protein + 1% DL-Methionine			0.72	
Beef			0.74	

1. Biological Value.
2. Net Protein Utilization.
3. Supplementation level 1.2 grams/16 grams nitrogen

From the standpoint of reducing the extent of protein-calorie malnutrition by improving the utilization of legumes, studies carried out in infants are more relevant than studies with older subjects. Graham (20) has evaluated the effects on nitrogen retention of supplementing soy protein with methionine. In his experiments methionine addition to the commercial formula Sobee, a soy isolate or full fat soy flour was studied. In addition retention on Sobee with or without added methionine was compared to retention on Pro-Sobee, a commercial formula enriched with methionine. As shown in Table 4 in all cases reported methionine addition had a consistent and beneficial effect on nitrogen retention in infants ranging in age from 6 to 23 months.

TABLE 4
METHIONINE SUPPLEMENTATION OF SOY PROTEIN STUDIES IN INFANTS[1]

Age (Mo.)	Protein	DL-Methionine mg/Kg/day	Days	N Intake mg/Kg/day	N Retained mg/Kg/day
21	Sobee	—	6	240	42
	Sobee	11	12	232	70
23	Sobee	—	12	194	23
	Sobee	14	19	198	54
13	ProSobee[2]	—	9	320	95
	Sobee	—	15	320	79
13	ProSobee[2]	—	16	317	105
	Sobee	22	18	320	106
6	Soy Isolate	—	10	238	12
	Soy Isolate	20	9	240	44
8	Soy Isolate	—	6	240	63
	Soy Isolate	20	6	240	88
15	Soy Isolate	—	9	239	27
	Soy Isolate	20	9	242	57
21	Casein	—	1	320	107
	Soy Isolate	—	5	320	77
21	Casein	—	9	320	137
	Soy Isolate	16	6	320	107
19	Full Fat Soy Flour	—	2	280	69
	Full Fat Soy Flour	20	20	282	109

1. Data of Graham (20).

2. ProSobee fortified with DL-Methionine.

A beneficial effect of methionine addition to soy protein fed to infants would be expected on the basis of the animal experiments (5). In the United States at the present time it is common practice to add methionine to soy formulae fed to infants. It is interesting to note that this practice antedated the studies of Graham and was apparently based primarily on the prior animal experimentation. At the present time one would have to conclude that the desirability of adding methionine to soy formulae has not been conclusively demonstrated. Fomon et al. (21) have convincingly shown that a methionine supplemented formula based on soy isolate with protein supplying 6.5% of calories when fed to 13 infants resulted in growth comparable to milk formulae supplying greater intakes of

protein. However in previous work, Fomon (22) has reported that a soy formula without added methionine supplying protein at 6.8% of calories when fed to four infants resulted in growth and nitrogen retention equivalent to that on human milk. The work of Graham is apparently the only research in which the effects of soy with and without added methionine have been directly compared. Such direct comparisons are necessary because of the many complications that result when different formulations are compared. For example Theuer and Sarett have demonstrated marked differences in the level of trypsin inhibitors present in a series of commercial soy formulae (23). Differences in the adequacy of vitamin and mineral levels in commercial soy formulae also have been stressed (24). In considering the need and level of methionine addition to soy formulae several additional considerations should be kept in mind. Any beneficial effect from methionine addition would likely be very difficult to demonstrate at higher protein intake. Also D-methionine is apparently not utilized to any significant extent by the human infant (25).

V. *Practical Implications*

In discussing the practical aspects of the amino acid supplementation of legumes in human nutrition two basic situations can be considered. These are 1) food staples of legumes and root crops in which case most of the dietary protein is supplied by legumes, and 2) food staples of legumes and cereals in which case the dietary protein is supplied by varying mixtures of cereals and legumes. The very low level of animal protein and importance of plant protein in diets in developing countries, especially for the poorest people has been stressed elsewhere (26).

Legumes - Root Crop Mixtures

When legumes are nearly the sole source of protein, methionine is clearly the first limiting amino acid by chemical score and by rat growth. This would also be true for mixtures of legumes with milk or meat. The nutritive value of legumes has been discussed in considerable detail elsewhere (27). Based on the work of Graham (20) and the observation that most commonly consumed legumes are lower in sulfur amino acids than soy, one would anticipte that methionine addition would be of practical value. Whether there is in fact a beneficial effect from adding methionine needs to be documented with well controlled studies in human infants.

Legume - Cereal Mixtures

In most developing countries the more common situation is for dietary protein to be supplied by a mixture of cereals and legumes. Based on food balance sheets for 13 countries legumes typically supplied 19% and cereals 81% of the plant protein intake. The nutritive value of such mixtures of cereals and legumes has been discussed extensively in previous publications (28-29).

Changes in amino acid patterns in varying mixtures of corn and beans are summarized in Figure 1. As the proportion of beans increases tryptophan becomes briefly first limiting but then with beans comprising 14% of the mixture sulfur amino acids become

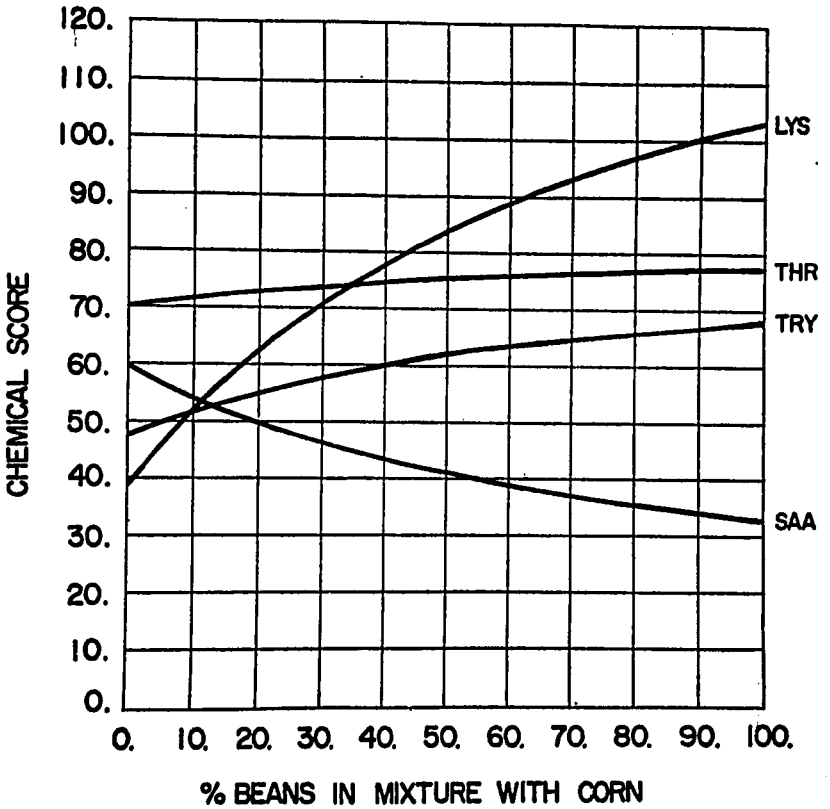

Figure 1. Chemical scores calculated for varying mixtures of corn and beans based on amino acid data in reference (5).

$$\text{Chemical Score} = \frac{\text{conc. of amino acid in mixture (mg/g nitrogen)}}{\text{conc. of amino acid in whole egg (mg/g nitrogen)}} \times 100$$

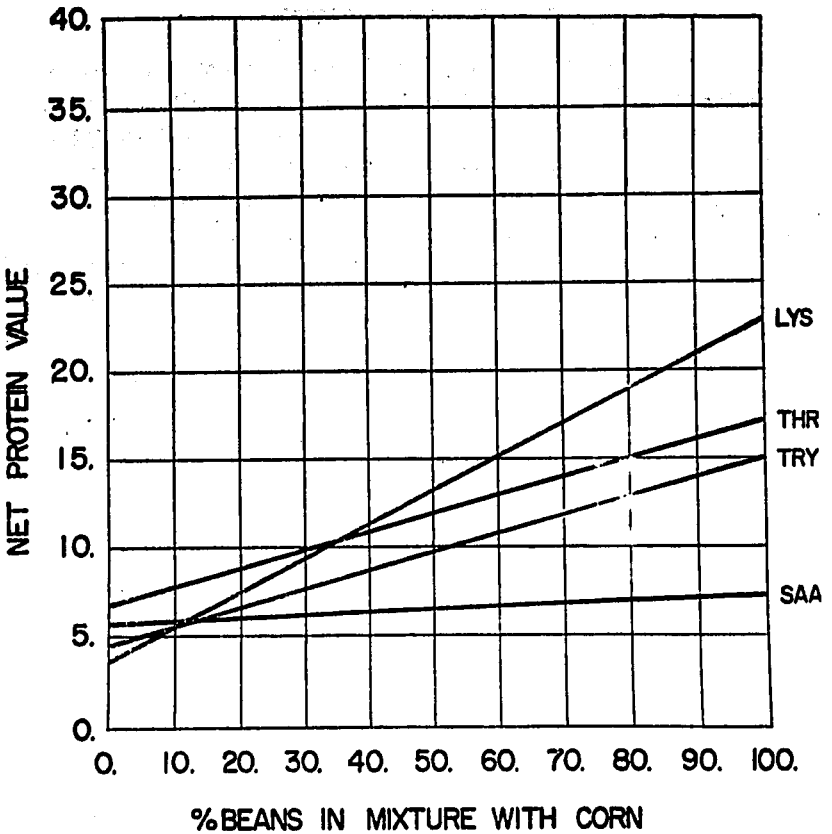

Figure 2. Net Protein Values (NPV) calculated for varying mixtures of corn and beans.

$$NPV = \frac{\text{Chemical Score} \times \text{\% Protein}}{100}$$

limiting. The net protein value (NPV) of a food mixture depends on the interaction of quality with quantity. The chemical scores for Figure 1 were multiplied by the protein concentrations and plotted as NPV's in Figure 2. This chart demonstrates that with less than 10% beans in the mixture lysine is limiting and with more than 14% methionine is limiting. This consideration does not take into account the problem of digestibility which, as has already been discussed is particularly a problem with beans. Also there are indications that whole egg contains about a 25% excess of sulfur amino acids as compared to the needs of the human infant (30, 31).

Nevertheless these calculations suggest that for corn-bean mixtures with the poorest protein value lysine addition would be more valuable than addition of methionine. With higher concentrations of beans more methionine should be beneficial, but not necessarily needed. Similar data are plotted in Figures 3 and 4 for mixtures of wheat of 70-80% extraction with chickpeas. In this case, however, lysine remains limiting until chickpeas comprise almost 30% of the mixture. In this case more lysine clearly should be of advantage in improving the protein value of the mixture and it is doubtful that methionine addition would play a useful role. These data may be of interest to both plant breeders and food technologists interested in protein improvement.

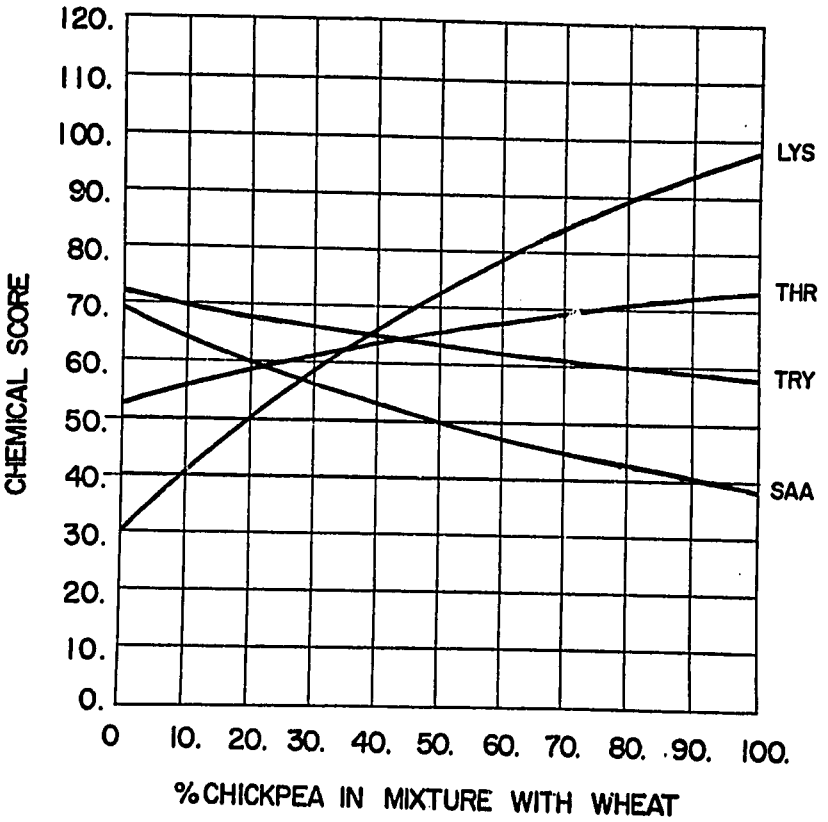

Figure 3. Chemical Scores calculated for varying mixtures of wheat (70-80% extraction) and chickpeas.

$$\text{Chemical Score} = \frac{\text{conc. of amino acid in mixture (mg/g nitrogen)}}{\text{conc. of amino acid in whole egg (mg/g nitrogen)}} \times 100$$

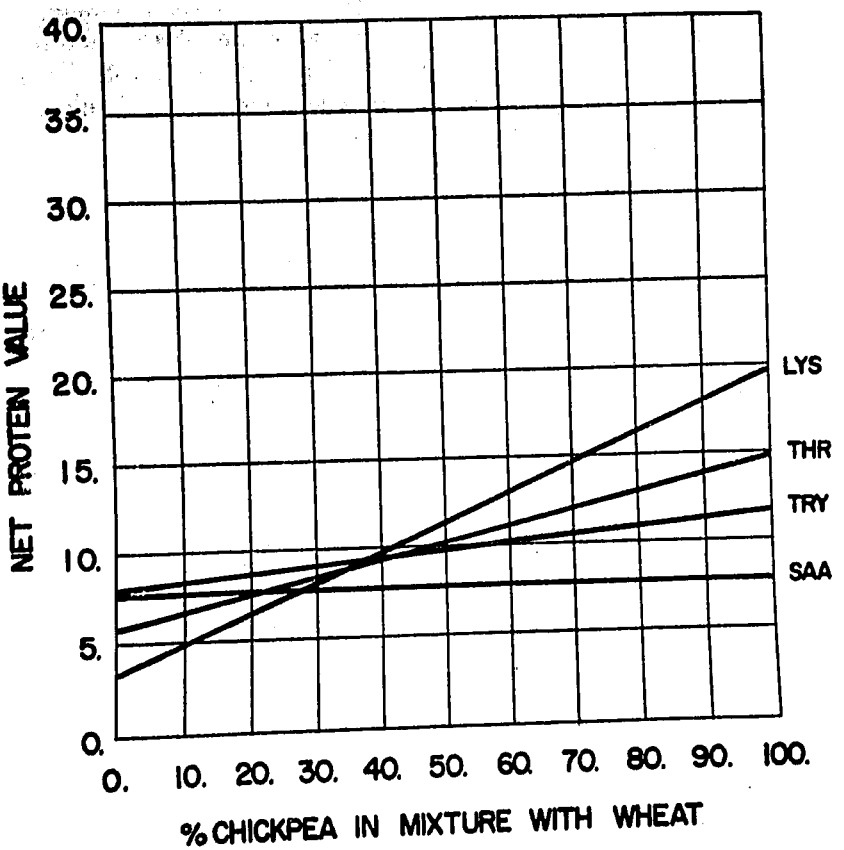

Figure 4. Net Protein Values (NPV) calculated for varying mixtures of wheat and chickpeas.

$$NPV = \frac{\text{Chemical Score} \times \% \text{ Protein}}{100}$$

The above considerations applied to legumes *per se* or theoretical mixtures of legumes and cereals. It would be of interest to look at chemical scores segregated by region and income within a large and diversified country such as Brazil. The Brazilian Institute of Economics has published the results of an extensive food consumption survey carried out in 1961-1963 (32). Within this report consumption data are reported by income level and by region. Using the data in this report amino acid patterns were calculated by the computer for three income levels and three major regions of Brazil. In Table 5 chemical scores calculated from these data are listed along with corresponding data calculated from the 1960-1962 food balance sheet for Brazil (33). The close correspondence between the values calculated from these two completely different sources of data is striking. The differences in chemical scores of these dietary patterns by income and by region are quite small. The data strongly suggest that for all incomes and all regions methionine is clearly the first limiting amino acid. Increasing the dietary supply of methionine should be of benefit in improving protein availability in Brazil. Mechanisms for accomplishing this include infusing methionine into beans or breeding beans with a higher content of

TABLE 5

CHEMICAL SCORES OF DIETARY PATTERNS IN BRAZIL[1]

Region	Family Income Cr/year	Chemical Score			
		Lysine	Sulfur Amino Acids	Threonine	Tryptophan
Brazil[2]	All	78	56	74	68
Brazil[3]	<100	82	54	73	82
	500	85	57	75	82
	>2500	89	59	76	83
Northeast[3]	<100	95	48	77	77
	500	94	55	78	80
	>2500	93	59	77	80
East[3]	<100	79	56	73	86
	500	83	57	75	84
	>2500	87	58	76	83
South[3]	<100	74	57	71	84
	500	79	58	73	83
	>2500	87	60	76	84

1. $\frac{\text{conc. of amino acid in diet (mg/g Nitrogen)}}{\text{conc. of amino acid in whole egg (mg/g Nitrogen)}} \times 100$

2. Data obtained from reference (33).

3. Data obtained from reference (32).

methionine. The data in Table 5 also support the suggestion that manioc flour be fortified with methionine as a means of balancing the methionine deficiency in beans (34). In view of the difficulties with legumes, especially poor digestability and the presence of toxic factors, these speculations have to be considered quite tentative until more data obtained in human subjects become available.

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SUMMARY

Rat studies which have evaluated the effect on growth of adding amino acids to a number of legumes have been reviewed. Addition of methionine to common beans, lima beans, hyacinth beans, peas, cowpeas, chickpeas and pigeon peas in all cases resulted in a protein capable of supporting good growth of the weanling rat. The pigeon peas required simultaneous addition of tryptophan. Although analogous experiments with human subjects have apparently not been reported limited data obtained on the methionine supplementation of soy protein fed to infants and children confirm a beneficial effect on nitrogen balance of methionine addition. Available data suggest that increasing the amount of methionine should be of value in human diets where most of the protein is supplied by legumes or legumes combined with small amounts of meat and milk. Calculations based on food survey data suggest that in Brazil methionine addition should be of value irrespective of region or income level. The necessity for more human studies is stressed.

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The alternatives in the complimentation and nutrification of legumes

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The protein quality potential of legumes in providing a very adequate basis for complimentation with cereals was recognized very early in the vegetable mixture studies of Bressani *et al.* (1) This idea of blending a cereal with a legume or oilseed can be promoted either by altering the existing dietary or by the development and introduction of products for supplementing the diet such as INCAPARINA. However, there is a practical realism about complimentation which is not often mentioned and that is the fact that it requires promoting changes in dietary habits (customs). *Optimum* complimentation requires a quantity of legumes which (a) is often not the usual dietary practice and therefore would require a change in buying and eating habits; and (b) would require an increase in purchasing power which is rarely available, e.g., beans cost more than corn or manioc or rice. These social and economic factors necessitate a further intervention, namely, and education program because the health significance to the consumer of the needed increase in expenditure is not immediately obvious.

As long ago as 1962 (2) demonstrated the feasibility of the complimentation of beans *(Phaseolas vulgaris)* with a cereal such as corn but the required protein ratio for optional response is approximatly 50-50, with is not the dietary custom. Further, depending upon how the ratio was skewed from this point the limiting amino acid differs; if the diet is more beans than corn then the limiting amino acid is methionine, otherwise it is lysine. More important if the commodity composition of diets, *i.e.,* the ratio of different foods used are considered in terms of actual practices (*e.g.* 20% Beans, 80% Cereal) the greatest nutritional benefit would be derived from increased protein alone. This emphasizes the need to first pay attention to technological or food practices which could increase *utilizable* protein *per se,* be it genetic breeding program for increasing the protein content of beans and/or the

introduction of other legumes, oil seed or cereal protein foods into the dietary. But how does one increase protein without radical changes in food habits?

Fortification with amino acids and/or whole protein or genetic manipulations which result in the protein quality improvement of an *existing* amount of a staple in the diet are apparent likely alternates because each permits capitalization on the existing dietary habits of the population. First let us consider genetic manipulation for increased protein. We must recognize that breeding programs (3) have to be directed to (a) a number of different varieties of legumes even though a few (30 out of 30,000) are much more commonly used than others, and (b) to breeding for increased protein in a plant product which as already protein-rich, and/or (c) to breeding for increased methionine/cystine levels if legumes are the plants in question and are being consumed with starchy roots or tubers. In all instances, these genetic improvements have to be realized without compromise in, and preferably with improvements in yield, adaptability, and consumer acceptance (*i.e.*, high organoleptic factors and low toxic and flatulence factors). Considering the foregoing and the current state of the art of legume breeding programs, the short range outlook is not encouraging. One must concur with the recent PAG statement (4) that the first order of priority in upgrading human nutrition through food legumes is genetic improvement in productivity, adaptability and yield stability; and only secondly in breeding for improved protein quantity and/or quality.

Fortification of the dietary and/or one or more processed legume or cereal products has a more immediate potential. The rationale of amino acid fortification as applied to legumes already has been discussed by Jansen (5). The decision as to whether fortification should be primarily associated with the legume, cereal or root/tuber portions of the dietary is not academic because it is the dietary as a whole that must be improved and therefore the selection of the proper food vehicle is directly dependent on determining the most consistently used food with which fortification technology is compatible. Again this type of intervention must be accomplished without altering food acceptance or habits. In the case of manioc flour, Dutra de Olivera *et al.* (6) have demonstrated the advantages of the methionine fortification of manioc rather than the common bean. In terms of immediate relevance, the current experience with the fortification of three cereals namely wheat (7, 8), rice (9),

and corn (10) should not be overlooked nor curtailed or minimized while awaiting advances in possible legume fortification technology.

As far as legume fortification technology is concerned, the only existing significant advancement in terms of known legume fortification technology is in the case of processed soybean flours such as fabricated in the USA and used primarily as meat extenders (11). These products as applied to school food service in the United States (12) have included micronutrient additions but not amino acids. It should be noted however that this technology of fabricating proteins is sophisticated and not immediately practical at the community level of developing countries, but could be applied to the development of simulated nutrified protein concentrate legume-like or legume containing products, but that technology needs more research and is not immediately relevant. Further, it must be acknowledged that as a point of intervention for amino acid fortificants and micronutrients, cereals and tubers or roots are more suitable because village or regional processing is frequently involved as a prerequisite to the preparation of these staple daily food products and therefore a site for the introduction of the intervention is more accessible; whereas, legumes are more often prepared in the home and a point of intervention is therefore difficult to identify.

A relatively recent processing development at a national level has been the use of some legumes in multifood mixtures manufactured for use as supplements or weaning foods (13). This approach which began with Incaparina and Pronutro in all probability was further stimulated by the production of Corn-Soy-Milk (CSM) from surplus US commodities (14). Cardaro & Call (15) have challenged the nutritional effectiveness of such foods with the possible exception being CSM because enormous amounts, two billion pounds since 1966, have been donated, and utilized, in obvious famine situations, or in compensation for work or school attendance situations. Note that the exchange for money was not involved. This experience with supplements which utilize the principles of complimentation and nutrification and frequently capitalize on indigenous protein sources must not be minimized because of questionable success. This does not negate continued emphasis on the development of protein mixtures. On the contrary it emphasizes the need to utilize the underlying principles involved to develop products which do not require a change in dietary habits which the existing supplements as now used do require. Dietary supplements have a place in situations of catastrophes where the people have

no choices and survival is the issue. Ironically, the products developed to date do not completely meet this type of application either, because they do not always include all the needed micronutrients for optimal protein utilization; but further nutrification of these balanced protein sources would not be difficult. When the goal of the intervention is to enhance the nutritional well being of an otherwise nominal populace, the new food must be a part of old food habits. For the urban consumer this may require commercially prepared higher protein legume/cereal/tuber or root food products, and for the rural consumer the introduction of fortification premixes or simulated kernels of protein concentrates and micronutrients.

Initial experience exists with the technology of fabricating proteins (11) and fabricating simulated kernels, e.g., rice (9) corn (10) and bean (16). With more technological experience, the suitability of such concepts at the field level would need to be critically examined. Given such a protein delivery system, it is essential that concomitant micronutrient needs to be considered. Nutrification - the incorporation into selected food products of limiting micronutrients based on protein in the product or the resultant diet use (17) would enhance the nutritive value and balance of the dietary.

If one pursues the concept of a nutrified protein fortification blend, the physical form to be mimicked would be dictated by (a) the food (staple) selected and (b) the home, village and/or commercial processing procedures applicable to the staple. It is self-evident, that if a food staple such as beans are selected, the physical form should probably be a synthetic bean, because beans are invariably prepared in the home. For those instance where commercial products are made with powdered beans, the mix would ideally be a powder.

One may wonder if the idea of a pot cube, such as proposed in the case of Nutricube (18) would not be viable. This is the nutrient blend in question, marketed as a universal supplement, in a nondescript form to be added to any number of food preparations particularly those involving a pot. The disadvantage in this concept is the same as for supplements in general - there is no garantee of equal distribution because it can be added to so many different foods and it has a greater risk because excess use is not avoidable.

There is no question of the need for protein and micronutrients in the populations in question. There is little advantage to only providing micronutrients, which act principally as coenzymes, if the utilizable protein of the diet is inadequate (19). On the other hand to increase utilizable protein without the concomitant provisioning of the *limiting* micronutrients for the optimal metabolism of protein is also foolhardy. In this respect Bressani (20) has shown in animals that minerals are often more limiting than vitamins in obtaining maximal growth response. It is difficult to justify a public health intervention based on a one micronutrient - one carrier system, such as the public health enrichment of sugar with vitamin A. True the intervention could diminish the incidence of vitamin A deficiency but the probability is that the recipient will then demonstrate a need for the next limiting micronutrient and the overall long range effectiveness of the initial intervention will be difficult to substantially improve over time. This is a debatable point but I believe experience in ongoing field trials (7) (9) (21) will demonstrate the potential long range implications of a *balanced* intervention to be significant in terms of morbidity, mortality and performance.

If one were to devise a *balanced* intervention mix for a region of a country such as Northeast Brazil where a need exists and the population uses beans in the diet every day —the protein—nutrification mixture should include a protein concentrate such as soy flour with added Lysine, Vitamin A, Thiamine, Riboflavin, Pyridoxine, Calcium and Iron. Incidentally since soy is grown in Brazil its systematic diversion to such a use may need technical as well as political emphasis.

It is slowly becoming evident that there is some universality in the nutrient deficiences in various countries and therefore in the nutrients which need to be used to intervene into the diet. It appears that, if the concept of a fabricated kernel, be it corn, bean, etc., is a plausible approach, then it would seem appropriate to study the technology of manufacturing such products *and* the suitability of the concepts in the village milieu. Given that demonstrated benefits ensue, there is a distinct possibility that the economics of a given intervention mechanism (e.g. fortification-nutrification of a cereal rather than of a legume, vice versa, or both) will determine the final choice. We must put aside estimates of cost based only on the cost of the nutrients. In reality, based on the experience with enriched bread and similar products in the USA, the cost of

ingredients are a small percentage, if only micronutrients are involved. If amino acids or protein are included then costs are significantly but not dramatically more. In any event, I estimate that one third to one half of the total product cost is for blending and/or fabrications, packaging and distribution. All interventions should also have to have some sort of quality control, i.e., a measure of continued effectiveness. Initially, this requires a simple monitoring program. The needed research to determine the cheapest and yet most effective distribution and quality control network has yet to be undertaken.

If and when total world protein supplies become more scarce, one must not overlook the feasibility of the combination of cereal and legume fortification and nutrification, in order to spare protein supplies by optimizing utilizable protein with limiting amino acids and micronutrients which are for the most part synthesized (22).

SUMMARY

The alternative approaches to the nutritional improvement of legumes must capitalize on the dietary habits of the populace and must first be directed toward increased dietary protein.

Breeding programs must be directed to increase yield and decrease adverse organoleptic, toxic and flatulence components before emphasis on characteristics for increased utilizable protein.

Whereas the principle of complimentation to enhance utilizable protein is sound, supplemental foods based on these principles have had questionable impact, because such products are not an integrated part of the dietary. However, the concept of complimentation when merged with amino acid fortification, nutrification and protein fabrication technology would permit the development of fabricated "supplements" which mimic a staple in the diet. Such nutraconcentrates which can be readily integrated into the distribution channels of that staple could be utilized without necessitating changes in diet habits.

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Clinical and experimental studies on common beans

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JOSÉ EDURDO DUTRA DE OLIVEIRA **

INTRODUCTION

It is well kown that common beans *(Phaseolus vulgaris L)* is one of the main sources of protein in the diets of many persons in different parts of the world (1). This is especially true in Latin America and particulary in Brazil (2). In Brazil the last food bälance sheet showed an average availability of 59.2g of beans per person. Several dietary surveys have also shown that rice is largely consumed along with beans (2, 3).

The consumption of rice and beans in different areas of Brazil and what they represent of the total protein and energy in the diets are shown in Table I.

Considering these data, along with the fact that protein-energy malnutrition is widespread in areas were these foods are included in the basic diet of children and adults (4), we started a series of studies on rice and bean protein, alone or in combination to bring out their best nutritive value.

Nutritive Value of Rice and Beans
Amino Acid Studies

The amino acid composition of the Brazilian largest consumed rice and beans varieties was determined by ion exchange chromatography with an automated amino acid analyser. The essential amino acids present in these foods are shown in Table II, where they

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are compared with eggs. It is again shown that the most deficien amino acids are lysine and the sulphur containing, respectivelv for rice and beans.

Biological Assay Studies in Rats

These results were later checked in animals, were PER studies in rats fed rice diet supplemented with lysine and threonine showed that the best results were reached with an additional 0.3% and 0.2% respectively of these amino acids (Table III). With a bean diet (Table IV) the best nutritive value was found with a 0.3% methionine supplementation.

The importance of the amino acid supplementation on the absorption and retention of nitrogen was also shown in balance studies in rats. These results are presented in Table V.

It can be seen that the supplementation does not interfere with the digestibility of the protein, but increases significantly its retention.

Nutritive Value of Combinations of Rice and Beans

The nutritive value of rice and bean combinations was also tested by the PER method. The best results were obtained with 50%, 60% and 70% of protein from rice and 50%, 40% and 30% from beans. This mixture has a better nutritive value than rice or beans alone, showing the complementation between the proteins of these two foods.

The mixture, 50% of the protein from beans and 50% from rice, was selected for further studies.

Long Term Studies With Rats

A long term study on reproduction and growth was carried out in rats, followed by determination in carcass of water, protein, fat and ash. A group of 3 adult females were bred and fed a rice and bean diet in 50:50 protein combination. A control group of similar animals were fed a casein diet. Both diets had the same 10% level of protein.

The offsprings of each rat were weighted and some of the newborn killed for body composition determination. Each rat was left with the same number of newborn. They were killed for study at 4 weeks or at 6 months. After weaning all animals where always kept on the same experimental or control diets.

With 3 months of age 1 male and 3 females of the first generation were bred again and followed the same experimental design.

TABLE 1
DAILY INTAKE OF COMMON BEANS AND RICE IN SOME BRAZILIAN CITIES

CITY	RICE (g)	BEAN (g)	%TOTAL PROTEIN INTAKE	%TOTAL CALORIE INTAKE
Palmeiras de Goias (GO)	243	91	64.2	48.2
Guaratiba (GB)	116	81	41.0	30.3
Juazeiro (BA)	40	61	26.6	16.0
Buritizeiro (MG	107	67	51.7	37.0

TABLE 2
ESSENTIAL AMINO ACID PATTERNS (mg/g ESSENTIAL AA)

AMINOACID	RICE	BEAN	EGG
Isoleucine	94(73)	100(78)	129
Leucine	188(110)	201(117)	172
Lysine	85(68)	141(113)	125
T.Aromatic AA	281(145)	273(122)	195
T.Sulphur AA	123(115)	46(43)	107
Tryptophan	79(80)	113(115)	99
Valine	121(86)	115(82)	141

( ) % In relation to egg protein

TABLE 3
ROTEIN EFFICIENCY RATIO OF RICE SUPPLEMENTED WITH VARIOUS LEVELS OF L-LYSINE AND DL-THREONINE

LYSINE (g%)	THREONINE (g%)	WEIGHT GAIN (g)	PER
None	None	37.2	2.35
0.1	0.1	63.7	2.94
0.1	0.2	63.8	2.86
0.1	0.3	58.5	2.78
0.2	0.1	79.7	3.32
0.2	0.2	78.7	3.29
0.2	0.3	80.7	3.27
0.3	0.1	83.8	3.59
0.3	0.2	91.7	3.71
0.3	0.3	91.2	3.71

TABLE 4

PROTEIN EFFICIENCY RATIO OF BEAN SUPPLEMENTED WITH VARIOUS LEVELS OF DL METHIONINE

METHIONINE (g%)	WEIGHT GAIN (g)	P E R
None	2.8	0.28
0.1	50.5	2.43
0.2	61.0	2.80
0.3	66.8	2.93
0.4	62.8	2.92

TABLE 5

NITROGEN BALANCE IN RATS FED RICE OR BEAN DIETS PLUS AMINOACIDS

DIET	N INTAKE mg/d	ABSORPTION % INTAKE	RETENTION % INTAKE	RETENTION % ABSORPTION
BEAN	82	71.3	35.6	49.9
BEAN + MET.	90	67.8	54.6	80.6
RICE	92	84.4	49.2	58.3
RICE+LYS+THR	90	85.0	64.3	75.7

TABLE 6
BODY COMPOSITION OF RATS FED CASEIN AND RICE AND BEAN DIETS 1st GENERATION

Body Comp. / Period / Diet	WATER * %			FAT * %			PROTEIN * %			ASH * %		
	BIRTH	4 WEEKS	24 WEEKS	BIRTH	4 WEEKS	24 WEEKS	BIRTH	4 WEEKS	24 WEEKS	BIRTH	4 WEEKS	24 WEEKS
CASEIN	82.4±1.3**	69.8±1.0**	61.1±0.2**	2.7±0.5**	10.6±2.4**	12.3±1.4**	11.2±1.1**	15.1±0.3**	20.3±1.4**	2.0±0.2**	3.0±0.1**	3.4±0.03**
RICE+BEAN 50:50	80.5±3.8	69.9±1.5	59.4±2.3	2.8±1.0	10.8±2.5	17.2±2.8	11.6±1.2	14.3±1.3	18.1±0.4	2.0±0.2	3.1±0.2	3.4±0.1

(*) WET BASE
(**) St.DESVIATION
MEANS OF 6 RATS

TABLE 7
BODY COMPOSITION OF RATS FED CASEIN AND RICE AND BEAN DIETS 2nd GENERATION

Body Comp. / Period / DIET	WATER* %			FAT* %			PROTEIN* %			ASH* %		
	BIRTH	4 WEEKS	24 WEEKS	BIRTH	4 WEEKS	24 WEEKS	BIRTH	4 WEEKS	24 WEEKS	BIRTH	4 WEEKS	24 WEEKS
CASEIN	83.7±1.9**	68.0±3.4**	59.3±0.1**	1.9±0.6**	13.2±2.0**	16.9±0.4**	11.0±1.4**	13.8±1.3**	17.7±0.1**	1.8±0.3**	2.7±0.3**	3.2±0.1**
RICE+BEAN 50:50	80.5±3.8	66.6±0.8	57.7±0.1	3.1±0.5	14.9±1.1	17.3±2.3	13.7±2.8	13.0±0.8	18.5±2.8	2.0±0.5	3.0±0.8	3.5±0.1

(*) WET BASE

(**) ST. DESVIATION
MEANS OF 6 RATS

Results of the experimental and control animals at birth, 4 weeks and 6 months is shown in Table VI. There is a change in the body composition with age in both groups, but not between animals of the same age group fed the rice-bean or control diet.

In the second generation, the body composition at birth, 4 weeks and 6 months were again similar for both groups at the same age, as one can see in Table VII. The weight curves of the animals for both generations are quite similar, with a small increase in growth for the animals fed rice and bean diets when compared to the control group fed casein.

Nitrogen Balance Studies in Normal Children

Considering the results of these animal experiments it was thought of importance to study the same rice and bean diets in children. Pre-school children of low socio-economic status without any actual diseases were fed a rice and bean mixture similar to the one fed to the rats in the long term studies. They were kept in a metabolic unit with 24 hours supervision. The protein intake was kept at the level of 1.5 g/k/day. Each one was kept on the same diet during 6 adaptation days and 6 balance days. We also tried to analyse the influence of the energy intake on the nitrogen metabolism, the results are shown in Table VIII.

TABLE 8
NITROGEN BALANCE IN CHILDREN FED RICE AND BEAN MIXTURE WITH VARIOUS LEVELS OF CALORIE INTAKE

CALORIES	N INTAKE	N EXCRETION		% INTAKE	
	mg/Kg/d	URINE mg/Kg/day	FECES mg/Kg/day	ABSORPTION	RETENTION
1.400	190	88	64	66.4	20.4
2.300	190	76	58	70.0	29.5
3.000	196	20	75	61.0	24.5

The absorption and specially the nitrogen retention was found at a good level even with a low level of calories in the diet. These results are higher than the ones found in children when they receive a mixture of corn and beans (5).

In conclusion the overall results both in animals and children have confirmed a fairly good nutritive value of a well balanced rice and bean mixture. A further advantage of this mixture is its conventional use by local people in different areas of the world.

SUMMARY

Common beans (Phaseolus vulgaris L) is the legume seed with highest consumption in Brazil. A supply of 59.2 g/person/day is said to be available Along with rice it is said to account for about 50% of the daily protein intake in the country. Beans are methionine deficient and rice deficient in lysine Mixture of them was shown to have a better nutritive value in rats when 30 to 50% of the protein comes from common beans. Further long term studies on the nutritive value of rice and bean mixtures were carried out on fertility and growing of young animals. Adult rats were fed a 50:50 rice and bean mixtures, and crossed during 2 generations with no differences between the animals of these groups and of a control group fed with casein. Parameters utilized were weight curves and body composition (water, protein, fat and ash). The same rice + bean mixture was fed to pre-school children and through nitrogen balance it was shown to have a reasonable good protein value. These results show again the effectiveness and good nutritive value of the rice and bean mixtures. Attention is called to the utilization of conventional foods on the improvement of local diets without changes in food habits.

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The Latin American Program for increasing yields of dry beans[1]

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Grain legumes and specifically dry beans are an important component of the diet in Latin America and throughout the tropics. As a source of protein it is particularly important in the diets of middle and low income groups where animal protein of any type may be scarce or practically non-existent. The crop is grown by subsistence farmers through much of the highlands and medium elevations in the zone, and may provide the principal source of quality protein for direct consumption on the farm.

Research and promotion in field beans has not been emphasized in the tropics to the same degree as commercial export crops (coffee, sugar cane, cacao, etc.) or even other staple grains (maize, wheat, rice, etc.). This was partly due to its lack of large scale production, and probably due to a lack of interest among researchers and planners in a crop with an apparent low yield potential and which is grown primarily by small farmers with little apparent potential to improve their economic or technological situation. The CIAT program in beans, which can really be considered a Latin American cooperative effort through a series of joint projects and regular meetings with national agencies throughout the zone, is dedicated to improving this potential.

1. Presented by C. A. Francis (CIAT Physiologist) in the IITA Grain Legume Improvement Workshop, October 30, 1973, Jbadan, Nigeria (in English).
Presented by G. Hernandez (CIAT Breeder) in NDBRC - BIC Meeting in the session on Bean Physiology. Nov. 7, 1973, Rochester, New York (in English).
Presented by A. Pradilla (CIAT - UV Metabolic Unit) in the Workshop on Nutritional Quality of Beans, November 6-9, 1973, Riberao Preto, Brasil (in English and Portuguese).
Presented by A. V. Schoonhoven (CIAT Entomologist) in the meeting of the American Society of Entomologist, Latin American Sesion, November 1973, Dallas, Texas (in English).
Presented by G. Galvez (CIAT Pathologist) in the meeting of the American Phytopathological Society, Caribbean Division, December 9-12, 1973, San Jose, Costa Rica (in Spanish).
C. A. Francis Coordinator-Physiologist and Breeder, Bean Production Systems Program, Centro Internacional de Agricultura Tropical (CIAT), Apartado Aéreo 6713, Cali, Colombia.

The overall purpose of the program is to increase the productivity production, and consumption of beans, to improve diets of low-income people and enhance the welfare of the subsistence farmer. Emphasis is placed on commercial beans as well as on associated cropping systems used by the small farmer, where there will be an expected "spin-off" or application of most results to commercial-sized operations as well. This program was launched as an integrated team in 1973.

Specific CIAT Objectives.

To guide our cooperative program with the many involved national programs and resolve the serious problems of low productivity and production, we are considering the following objectives:

1. Support national programs and other institutions concerned with bean research, development and promotion, through training and team organization and orientation.
2. Improve the yield potential of existing germplasm through collection, evaluation, recombination and testing of promising germplasm, particularly in the lowland tropics.
3. Increase the range of adaptation of new varieties with a genetic flexibility to variations in soil type, moisture, temperature and photoperiod.
4. Improve or maintain the protein quality and nutritional value of beans for their use in direct human consumption.
5. Develop genetic resistance to pathogens and insects as an effective and economic method of control, and the integration of this genetic material into a total control package.
6. Identify, reduce or eliminate the limiting physical and socio-economic factors which influence the production, marketing and consumption of beans.
7. Study, develop, test and demonstrate production systems under different levels of technology which can effectively increase economic yields over time and space of existing and improved varieties of field beans.

Professional Team in CIAT

The team of specialists now working in CIAT is divided into eight research groups. These are presented to provide a reference to persons wishing to communicate with us directly or to establish cooperative work with any specific group.

1. *Agronomy:*
   Dr. Reinhardt Howeler, Leader (Soils Trials, Carimagua)
   MS. Carlos Flor (Agronomic Trials, Outreach)
   I. A. Carlos Tamayo (Agronomic Trials, Palmira and Popayan)
   I. A. Carlos Gonzáles (Agronomic Trials, La Selva and Turipaná)
2. *Breeding:*
   Dr. Guillermo Hernandez-Bravo, Leader
   I. A. Rigoberto Hidalgo (Germplasm Bank)
   I. A. Alvaro Ramirez (Germplasm Evaluation and Breeding)
   I. A. Alberto Robledo (Germplasm Evaluation and Breeding)
3. *Economics:*
   Dr. Grant Scobie, Leader (1973)
   Dr. Per Pinstrup-Andersen, Leader (1974)
   M. S. Mario Infante
4. *Entomology:*
   Dr. Aart van Schoonhoven, Leader
   I. A. Alvaro Melendez
5. *Microbiology:*
   Dr. Peter Graham, Leader
   I. A. Victor Morales
6. *Nutrition:*
   Dr. Alberto Pradilla, Leader
   I. A. Luz H. Betancur (Aplied Nutrition and Agronomy, Turipana)
   Dr. Luis Fajardo, Universidad del Valle
   Dr. Francisco Linares, Universidad del Valle
7. *Pathology:*
   Dr. Guillermo Galvez, Leader (Virus Diseases)
   I. A. Jose J. Galindo (Fungus Diseases)
   I. A. German Alvarez (Bacterial Diseases)
8. *Physiology:*
   Dr. Charles A. Francis, Leader (Coordinator, Bean Prod. Systems Team)
   I. A. Francisco Motta (Growth Trials and Adaptation)

Research in Progress

Each working group within the Bean Production Systems Team has developed a research plan for the current year and the near future. The work in products is presented in a general form, and we invite any comments or suggestions from other colleagues in these areas. It is of particular interest to us to know who else is working with similar problems, and the CIAT team is extremely receptive to proposals for joint research projects.

1. *Agronomy*

The most important function of the production agronomist is to integrate existing information and components into an efficient technological package for the farmer. We are interested in determining for existing varieties, maximum potential and also the economically optimum yields which are possible through proper spacing, fertility and pest control. This will help to guide our physiology and breeding work to overcome the existing yield plateau, and more production up to levels previously out of reach. The concentration on small farmer systems, and the use of multiple species and crops stimulate not only studies of this system on the farm, but also trials which attempt to optimize the use of resources and time in bean production. Optimum combinations of populations of maize and beans, and eventually other crops as well, must be on other operations such as weed control, insect and disease control, and optimum use of fertilizer for a mixed cropping situation are generally unavailable, and must be developed.

The unique problems of acidity and infertility of the soils in the eastern plains of Colombia, coupled with the long-term agricultural potential of the zone, justifies some intensive investigation of agronomic practices for a maximum bean productivity for those soils. Similar types of soils cover large areas in Venezuela as well as Brazil. Initial trials show that black-seeded ones grow and produce better than other types. Cowpea seems particularly adapted to the zone. There is a challenge to the team to develop a profitable package for this zone.

2. *Breeding*

The major emphasis during the first year has been to bring together existing collections of *Phaseolus vulgaris* from around the world, and to evaluate this material in several locations. Of the 10.000 collections now in CIAT, more than half have been tested

in the Cauca Valley, and some 1500 have been tested in two additional sites. We are currently standardizing terminology and preparing a computer format and program to allow us to publish the first summary to date in early 1974. These collections will be available at that time, and the catalog to be published each year will be the best source of information on these collections and their agronomic and resistance characteristics. Data from these initial observations will be used to choose the potentially most productive lines of varieties over a range of climatic conditions, and to thus choose parents for the first crossing program later this year. Parents will also be chosen for their potential contribution to an ideal plant type, including high pod number and seed number per pod, seed weight/100 grains, top root tendency, strong stem, intermediate height (for monoculture) and strong climber (for planting with maize). The next year's program will involve continuing the germplasm bank, observations of advanced generations of selected materials, and generation of many new crosses on the basis of new concepts in plant type. The overall objective is to produce improved bean populations for the lowland tropics.

3. *Economics*

The work in economics is focused on identifying factors limiting production, productivity, distribution and consumption of beans, estimating their economic importance and predicting the likely impact of reducing or eliminating the effects of any one of these factors on such variables as farm returns by farm size, nutrition, employment and consumer real income. Furthermore, the economics group assists other team members in the economic evaluation of experimental results.

The primary purpose of the above work is to provide biological scientists in beans with information on the relative importance of alterantive research efforts.

Present work includes the collection and organization of available data on production, productivity, foreign trade, etc., in beans in order to establish a statistical base, furthermore, attempts are made to construct an economic model for the Colombian beans sector that will predict the effect of exogenous changes (e.g. agricultural technology) on: 1) Demand for labor and other inputs, 2) producer incomes, 3) producer and consumer nutrition, and 4) distribution of benefits from new technology. The model is expected to identify

the limiting factors and draw attention to those variables for which additional information is required.

The economics of bean production in various cropping systems will be studied in the near future. Likewise, attempts will be made to improve the understanding of factors determining consumer preference for beans, e.g., how do bean type, structure, color, etc., influence consumer preference?

4. *Entomology*

The emphasis here is again on genetic resistance, particularly to *Empoasca* sp., spider mites, *Diabrotica* spp., and white fly. Insect damage on beans is severe, and the problems of vectors for virus diseases make this genetic resistance in combination with virus resistance even more important in our search for a potentially productive new series of varieties. The majority of the screening work will be carried out in collaboration with national programs, where a particular insect is serious. As a part of the integrated control package, this genetic resistance will be combined with economically profitable insecticide recommendations and cultural practices to minimize damage to the crop. Additional activities which add to our understanding of the insect problem include studies of insect populations through the year as influenced by climatic changes, and the evaluation of losses due to insects in commercial crops of beans. The challenges of insect control in a multiple croping system are unique, and open entire new frontiers of research and eventual recommendations to the small farmer.

5. *Microbiology*

Nodulation and nitrogen fixation by legumes is highly dependent on the presence of a proper inoculum, either natural or introduced, and the nitrogen status of the soil. The initial work in CIAT has concentrated on collecting and evaluating a range of *Rhizobium* strains across varieties of beans for potential use as inoculants. The interactions of variety strain are impressive, and vary with temperature and nitrogen level. Tests on different sources of peat and appropriate sticking agents have given us critical data on the process which will eventually be put into commercial application when it is economically justifiable. The relationship between energy status of the plant, flowering and fruiting, and nodule retention provides the physiologist and microbiologist with an area of cooperative research which may lead to greater nitrogen fixation and increased production.

New and more productive plant types which will be developed through cooperative research efforts must also be examined in terms of nodulations potential and nodule retention, if that characteristic proves valuable. Nodulation on the top root vs. lateral roots was observed to vary among the bean collection tested, and their importance must be examined. Finally, the association of beans with maize and the possibility of the importance of the beans' nitrogen-fixing capability in promoting an increased availabality will be examined.

Nutrition

The nutritional package for the small farmer in Latin America often includes beans and maize, or beans with some other energy source such as cassava, potatoe or rice. The nutritional unbalance caused by cereal overeating as a result of the "Green Revolution" has been shown by Han *et. al.* in Korea. It becomes necessary to extend the "Revolution" to other staples at least maintaining present nutritive value. In attempts to improve the nutritional quality of pulses, the crop must be considered as only one component of the diet and should be selected in a direction to improve the total value of the diet. Quality of bean collection in the bank will be determined, initially for protein content, sulfur and/or sulfur containing aminoacids. Complete aminoacid analysis is made on those lines selected for valuable agronomic characteristics. An early emphasis is on quick screening methods, either visual or colorimetric, which will alow more rapid evaluation of a large number of genotypes. At present it may not be necessary to breed for better quality but it is important not to breed against it. The problem of toxic factors must be studied and solved by the most competent scientists available, and CIAT will collaborate directly in this effort.

Cooperative projects with Swine Program and the Metabolic Unit are evaluating other food grain legumes with respect to their nutritive potential. These projects are also coordinated with national and international centers in charge of the specific legume research.

Applied nutrition projects involving both maize and beans are underway as part of integrated team approaches to community development.

Some of the difficulties in combining nutritive value with better agronomic characteristics were described by Porter in his thesis dissertation. A highly significant negative correlation was found in the percent of protein and sulfur as a percent of protein. Yield and

percent protein were negatively correlated, the inverse correlation being greater at smaller yields than larger ones. In commercial varieties seed weight tended to be also negatively correlated with percent protein, sulfur content, and sulfur as percent of protein.

In other studies complete aminoacid analysis on the two hour hydrolizate of lines of beans, cowpeas and soybeans demonstrated the large variation of essential aminoacids among them. Some of this variations could, in part explain the low quality values encountered in legumes or oilseeds when only sulfur containing aminoacids are considered. Low lysine contents represent a danger when legumes or oilseeds are used in mixtures with cereals to compensate them. Protein Efficiency Rations (PER) tested for the same lines grown in the same locations and harvested in different years did not demonstrate any difference between seasons. There was a significant difference among the different lines tested. It is discouraging to realize that the two varieties with higher market price and public acceptance (Mortiño and Cargamento) are the ones with the worst nutritional qualities.

The sum of methionine and cystine and the sulfur content are both good predictor of PER values. The first is a better predictor but the relative ease of determination of the sulfur makes it a valuable tool in determining protein quality in legumes.

The results of this workshop will provide valuable guide lines for future collaborative research.

7. *Pathology*

The most important activity during the first year and continuing indefinitely is screening for genetic resistance to the most economically important diseases in Latin America: bacteriosis, rust root rots and the virus complex. Screening for resistance to other pathogens will necessarily be carried out in a number of cooperative proje_ts with research groups where such diseases occur. An organizational meeting has been held to determine where various projects are already under way, and how these may best be coordinated to avoid duplication of efforts and assure progress toward common goals. The unique disease problems of associated crops present an additional challenge. The production of disease-free or "clean" seed is another top priority project, carried out so far in the greenhouse, but soon to be moved to a new screenhouse facility. In early 1974 this will be extended to the field, in a

naturally dry zone far from any other legumes and possible vectors. Additional activities in progress include the economic evaluation of losses due to diseases, interaction of cultural practices and environment with incidence of disease, and evaluation of the economic feasibility of chemical fungicides, especially the new available systemics.

8. *Physiology*

The key to increases production potential may be found in a plant type which provides a greater number of fruiting modes, pods per node and seeds per pod and in a variety that can stand up under this load. The physiological effort is initially directed toward understanding the plant's growth and dry matter partitioning, and then toward how to increase economic yield. Through a cooperative effort organized with Cornell, Michigan State, and Hokkaide Universities, the most important areas of research in physiology will be taken on in an integrated manner. Exchange of germplasm, students, post doctoral fellows, and information will speed this process. CIAT will collaborate in a series of simplified growth trials over many locations in cooperation with national programs. In evaluations of the germplasm collections in CIAT, special emphasis is placed on yield potential and the morphologic characteristics which seem to contribute to this potential in association with maize. The apparent adaptive superiority in the tropics of black-seeded beans over other types must be explored, and the possibility of changing seed coat color through chemical mutagenesis may be useful in puttings this yield potential into acceptable types. Adaptation of beans to zones which differ in photoperiod and ambient temperature is being studied in the field in the tropics. This is a need to standardize measurements and terminology used to describe the bean plant, and a cooperative project currently under way will resolve this problem by pulling together the ideas of those now working in beans in the Americas and drawing a consensus from the group's response.

International Activities

The real results and benefits of CIAT's research and development will be expressed through advances and contributions of national research teams to the farmer in their countries. Thus, the international focus of our program and importance of the collaboration with other agencies cannot be stressed enough. The

following specific activities are all designed to give a maximum contact of our program with those in Latin America, and form the basis for our potential effectiveness.

1. Program organization and evaluation have been requested by several national programs, and we consider this a high priority among our other activities. This is particularly important when a new program is being organized and priorities established.
2. Training is central to our concept of international collaboration, and considerable time and energy is dedicated to the young scientists who spend from three months to one year in our program. These researchers are the future of their respective national programs, and a close working relationship with them will strengthen our capability to efficiently exchange germplasm and ideas, and to conduct cooperative projects. During the first year of activities, young scientists have been trained in Bean Production Systems.
3. Annual workshops for field research people have proven valuable in other crops as a dynamic activity which promotes both individual and group interaction. The informality of a workshop which emphasizes round table discussion and personal exchange leads to a mutual confidence and sharing of information and breeding materials which is often more valuable than the organized program. The site of this meeting will be rotated among countries, and organized in cooperation with national program.
4. Special topic workshops are planned for the next three years, and will emphasize the importance of an integrated team approach to research. The tentative schedule and titles are as follows:
   1974: Physiologic, Agronomic and Soil Problems Related to Beans.
   1974: Nutritional Implication of Mixed Cropping Systems.
   1975: Advances in Plant Protection in Beans.
   1975: Problems in Bean Breeding and Germplasm Evaluation.
   1976: Economic Implications of Increased Bean Production.
   1976: *Rhyzobium* Workshop Related to *Phaseolus* spp.
5. The publication of an informal newsletter with details on available publications, meeting, personnel currently working in national programs, and research abstracts is an ef-

fective way to maintain communication among researchers between the annual workshops.

6. The dynamic germplasm bank in CIAT will be catalogued, and an up-to-date description of seed availability and agronomic data provided each year. Within another year, a catalog of improved selections and advanced generations of crosses will supplement the one in original collections. Seed of either bean collections or improved lines will be available to national programs, seed companies, national universities, and other interested groups.
7. An abstracting service in both English and Spanish will be provided to bean research workers and libraries in the zone. This will supplement the bibliographical service already available from Turrialba.

Conclusions

The CIAT program for the improvement of the productivity and quality of beans is new, and the research priorities are under continuous review.

We depend directly on national programs for review of our own program, and attempt to provide the training, germplasm, and results which they find most critical to their progress in research and development. The CIAT Bean Production System Program is designed and staffed to serve national programs and collaborate in their efforts to increase production cooperative projects in research and training with universities and other organizations outside the tropics are also integral to our program, but are focused directly on the potential benefits of these contacts to developing tropical agriculture.

There is a strong potential pay-off for temperature programs in terms of availability of germplasm and locations for tropical training of students, and this incentive has already led to several mutually valuable projects. We invite participation in this effort to increase bean production in Latin America, and to help us reach a goal which is central to food supply in areas where malnutrition is still serious.

Carbohydrate digestibility and flatulence activity of beans

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INTRODUCTION

It is well known that beans *(Phaseolus vulgaris)* and soybeans *(Glycine max)* cause flatulence in human subjects. However, it has not yet been established what might be the cause of flatulence. At first the carbon dioxide of flatus was thought to originate from the bicarbonate of the pancreatic fluid. Later investigations of Steggerda and co-workers, in which a mixture of antimicrobial drugs was used, indicated, however, that gas production by beans in vivo and in vitro could be largely inhibited (16, 12, 11, 10). This result is only compatible with the view that a microbial process of gas production in the intestine is involved.

Carbon dioxide is not the only component of flatus. *Figure 1* shows the composition of the flatus of a test subject (7). Carbon dioxide and hydrogen are always present; in some subjects carbon dioxide prevails, in others hydrogen; methane may be completely absent (7).

Table 1 shows that each individual may react to a bean meal with gas formation in a more or less different way. Carbon dioxide and hydrogen are predominantly formed by anaerobic organisms in the ileocecal region; methane is rather a product of aerobic organisms in the colon. Nitrogen and oxygen originate from swallowed air. Figure 1 furthermore shows that peak gas egestion appears 5 to 6 hours after consumption of the bean meal.

Gas production in the gut is not only detectable by measuring the gas that passes the rectum; the gases partly diffuse through the intestinal wall and are transported by the bloodstream to the lungs, to be ultimately expired with the breath. Intestinal hydrogen gas leaves the body through breathing some 30 minutes earlier than it does as rectal gas. However, this convenient method of

detecting intestinal gas formation stresses too much the formation of the hydrogen component of intestinal gas, while carbon dioxide formation does not parellel hydrogen formation.

Which components of beans are fermented in the gut and give rise to flatus?

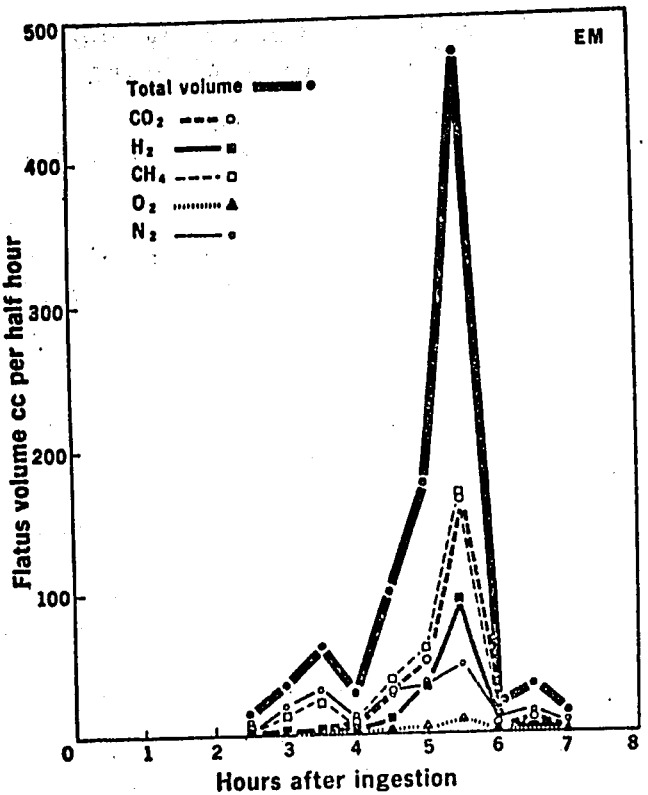

Figure 1. Composition of flatus, California small white beans, 100 grams (lit. 7)

In attempts to isolate and identify the gas causing factor in dry beans by successive physical and chemical treatment, and testing each successive fraction by feeding it to human subjects and measuring the resulting breath and flatus gas, Murphy (8) found that the factor is extractable with 60 per cent aqueous ethanol. Thus the factor has to be sought in the low molecular weight components. The bean fraction which still carries the gas-producing activity was found to contain a variety of substances among which some are not

yet identified. Attention focused on the soluble galactosido-oligosaccharides raffinose and stachyose (sugars built up of one, respectively two galactose and one sucrose unit) present in the active fraction. These sugars are not hydrolyzed and resorbed in the small intestine. They thus form a substrate for microbial fermentation in lower parts of the gut.

TABLE 1
COMPOSITION OF FLATUS AT PEAK GAS PRODUCTION

Subject	CO_2	H_2	CH_4
EM	33	19	29
WD	15	45	--
BF	10	44	11

Rockland et al. (13) investigating gas production by the intestinal anaerobe *Clostridium perfringens* in a synthetic medium found, however, that these sugars did not stimulate gas production. Accordingly they concluded that the primary stimulant in dry beans is something other than one of these simple sugars.

Rackis et al. (10) investigating gas production by an in vitro method of various soybean meal fractions by incubating them with cultures of anaerobic microflora of the dog's colon also found that raffinose and stachyose only produced small quantities of gas. These sugars, however, hydrolyzed in the component sugars, produced large amounts of gas (17, 14).

Investigating the flatulence activity of various soybean fractions in man, Steggerda (16, 10) concluded that the flatus producing factor does not reside in either the protein or in the high molecular weight fraction, and that only the low molecular weight meal constituents produce flatus.

In a later study, Murphy et al. (9) came to the conclusion that raffinose and stachyose fed alone at levels found in California white beans did not increase the carbon dioxide level of flatus, this finding being in agreement with earlier experiments in which stachyose was fed to human subjects resulting in a significant rise in the hydrogen level of the breath but no increase in the carbon

dioxide level of egested flatus (8). Thus, these bean sugars make a major contribution to the hydrogen component of the breath and flatus during bean flatulence and the major factor(s), which is responsible for the increase in carbon dioxide volume, remains unidentified.

That the origin of hydrogen and carbon dioxide in flatus differs, appears also from experiments with certain antimicrobial drugs, as the hydrogen component of flatus was more reduced than the carbon dioxide component (7).

Some more facts may be mentioned as being against the view that the galactosido-oligosaccharides are the sole bean components which are responsible for flatus.

Navy beans, for instance, contain only half as much raffinose and stachyose as soybeans, yet they produce twice the volume of flatus (8). Carbon dioxide in flatus increases four-fold with the doubled dose of soybeans, but methane and hydrogen remain unchanged (1). Soaking and cooking soybeans cut the raffinose and stachyose content by about 50 per cent (15); yet cooking in plenty of water has never been proposed as a means of reducing flatulence activity of beans. Although alcohol extracted whole white beans are less flatulent than the control sample, breath hydrogen is not diminished by extraction (1).

Summarizing it can be said that most investigators fairly agree in their conclusion that the flatulence principle must be sought in the soluble low molecular weight carbohydrate components of dry beans and soy products, and not in the high molecular weight fraction. This especially applies to the hydrogen component of flatus, but it is not quite certain whether it also holds for the carbon dioxide component. The question arises what might be the fate of the other indigestible components of beans in the intestine.

So far about the American investigations.

When starting our investigations on flatulence activity of beans (4) our leading hypothesis was that all carbohydrates escaping digestion and absorption in the small intestine are liable to bacterial attack in the large intestine, giving acids and gases as fermentation metabolites.

In our experiments we used Dutch brown beans with a starch content of approximately 48 per cent. In preliminary experiments with rats, the intestinal gas volume was measured. In addition to the effect of beans in the diet on intestinal gas volume, we determin-

ed the acceleration of food passage through the intestine of rats caused by beans that replaced the wheat starch in their ration.

From these experiments the following conclusions were drawn:

Cooked beans in the diet of rats both accelerated food passage and increased intestinal gas volume, and these effects became more pronounced with a higher percentage of beans in the diet. These phenomena did not disappear when the beans had been cooked for a longer or shorter period of time or after retorting.

Velocity of food passage was greatest with beans cooked only for a short time; the influence of cooking time on gas volume was not clear. This experiment could be related to determinations of the enzymatic digestibility of bean starch, when the cooking time was varied. Raw bean starch was digestible for about 20 per cent, the digestibility increasing with cooking time. Thus, in insufficiently cooked beans, a greater percentage of the starch passes the ileum undigested and is fermented in the colon.

Measuring the *in vitro* digestibility of the starch of soft cooking beans showed that some 10 per cent appears to remain difficult to digest, this percentage increasing to about 15 per cent for hard cooking beans (some of these beans not cooking to softness at all). However no difference was found either in the passage time of the food or in the gas formation for the two lots of beans. Obviously, the difference in starch digestibility is too small to be of any influence on fermentation in the intestine of rats.

Investigating the influence of whole beans, deskinned beans, and the cuticles on the passage time of the food, it appeared that the accelerating influence of beans on the passage time through the digestive tract of rats is mainly caused by the cotyledons and only to a minor extent by the cuticles.

We also investigated the influence of extraction with aqueous ethanol in the following experiments:

Cooked beans were dehydrated and ground to a powder, this powder being extracted twice (2 to 3 hours each) with 60 per cent alcohol under reflux. In two experiments with rats, the passage time of rations with the extracted beans and with the extracts were compared with that of the basal ration (Table 2).

It appeared from these experiments that the extracted beans and not the extract cause acceleration of food passage in rats. The influence of alcoholic extraction of beans on intestinal gas volume of rats was measured in three experiments (Table 3).

TABLE 2

PASSAGE TIME OF FOOD IN RATS WHILE ON THE BASAL RATION, ON A RATION WITH EXTRACTED BEANS AND ON A RATION WITH BEAN EXTRACT

Ration	Passage time (in min)	Passage time (in %)
Experiment I		
Basal ration	258	100
Ration with extracted beans (30%)	209	81
Ration with bean extract	265	103
Experiment II		
Basal ration	314	100
Ration with extracted beans (40%)	239	76
Ration with bean extract	312	99

TABLE 3

INTESTINAL GAS VOLUME OF RATS ON A BASAL RATION, AND ON RATIONS WITH WHOLE BEANS, EXTRACTED BEANS AND BEAN EXTRACTS

Ration	Average intestinal gas volume (in ml) Expt. 1	Expt. 2	Expt. 3
Basal (without beans)	1.02 ± 0.18	1.02 ± 0.18	1.02 ± 0.18
Whole beans	1.91 ± 0.21	2.49 ± 0.14	2.28 ± 0.15
Beans, extracted	1.73 ± 0.16	2.25 ± 0.16	2.20 ± 0.09
Bean extract	1.20 ± 0.15	1.47 ± 0.17	1.45 ± 0.08

The gas volume on a diet with extracted beans remained below the values obtained with whole beans; the extracts showed a minor activity.

Measuring flatus volume in three ambulant human subjects, we found the effect of alcoholic extraction to be less clear (Table 4).

TABLE 4

FLATULANCE ACTIVITY OF BEANS, EXTRACTED BEANS AND THE BEAN EXTRACTS IN 3 HUMAN TEST SUBJECTS

and the bean extracts in 3 human test subjects.

	Flatus volume in ml/24h per 100 g of beans		
Test subject	A	B	C
Blank	200 ± 40 (6x)	130 ± 20 (8x)	<50 (5x)
Brown beans	300/430	255/310	-
Extracted beans	250/215	185/300/370	50/90
Bean extract	200 ± 40 (2x)	130 ± 20 (3x)	<50 (2x)

Extracted beans showed a minor activity in test subjects A and C; only test subject B reacted to extracted beans with a response comparable to flatulence on unextracted beans. The extracts themselves showed no activity.

Our experiments were criticized by others, who considered the extraction procedure as not being exhaustive enough. This we may admit; nevertheless, the first extractions must have given the greatest yield.

From these experiments we concluded that, although the extracted components may have some activity, the greater part of activity was shown by the insoluble high molecular weight components.

Our attention reverted to the insoluble part of the indigestible carbohydrates. By subtracting water, protein, fat and ash content of dry beans from 100 per cent, the total calculate carbohydrate content amounts to about 60 to 65 per cent. The starch content, determined by a treatment with pancreatin, is approximately 48 per cent, thus the calculated indigestible carbohydrate content of dry beans amounts to about 12 to 17 per cent. This indigestible part may be increased by some 10 to 15 per cent of the starch, which is not easily digested.

Of the indigestible carbohydrates, about one fourth is soluble in water; it consists of some pectin and the galactosido-oligosaccharides.

The insoluble indigestible carbohydrates consist of the cell wall constituents cellulose, hemicellulose, and lignin, often called crude fibre, bulk or roughage. However, the usual chemical crude fibre determinations are quite unphysiological.

We next developed a simple method for the determination of true indigestible insoluble residue after successive treatment with pepsin hydrochloric acid and a buffered slightly alkaline pancreatin solution (5).

The analytical results show that the enzymatically determined crude fibre content of some types of legumes far exceeds the chemically determined crude fibre percentages (Table 5).

TABLE 5
INDIGESTIBLE RESIDUE AND CRUDE FIBER OF FOUR TYPES OF PULSES

	indigestible residue	crude fiber
	(percentage on dry matter)	
Brown beans (pressure cooked, total)	15.0	2.8
White beans (canned, decanted product)	15.7	2.4
Dun peas (marrow-fat) (canned, decanted product)	19.6	2.9
Peas (canned, decanted product)	13.2	2.1

For defatted soyflour, the discrepancy is somewhat less pronounced, the crude fibre content being 6.8 per cent and the indigestible residue being 11.7 per cent.

A question has to be raised as to what may be the fate of these indigestible components in our body after ingestion of a bean meal. To answer that question we have analyzed the faeces of four test persons eating 150 g of dry beans daily during an experimental period of one week. The content of lactic acid and of the lower fatty acids in their faeces were found to have increased. This proves that fermentation had taken place somewhere in their intestine. Stool weight was increased due to these acids promoting food passage in the large intestine. Only small percentages of starch and of the enzymatically determined cell wall constituents were found back in the stool. The components of crude fibre - together

with pectin and the galactosido-oligosaccharides —are not digested and absorbed in the small intestine. Hence their disappearance in the stool is only consistent with the explanation that they must have been fermented in the ileo-cecal region and in the large intestine, forming acids and gases as products of microbial decomposition.

Discussion

It may well be that fermentation of the low molecular weight galactosido-oligosaccharides takes place higher up in the small intestine, and that fermentation of the high molecular weight carbohydrates takes place in lower parts of the gut (pectin and hemicellulose are more easily fermented than cellulose; lignin is not fermentable). Thus a shift may occur depending on solubility and fermentability of the indigestible components by the different types of micro-organisms present in the various regions of the intestine, producing various acids and gases as fermentation metabolites.

High bacterial population densities in the small intestine are to be considered as abnormal (3, 6). Thus a rapid response (after half an hour or even shorter) with gas formation after consumption of a bean meal (8) may also be considered as abnormal. High bacterial counts in the small intestine can be found some hours after food consumption (2) largely increasing gas formation (11).

To obtain a link between the American investigations and our work concerning fermentation originating from the cell wall polysaccharides, it would be relevant to test the insoluble residue of beans obtained after pretreatment with pepsin and pancreatin in human subjects before breakfast.

Most often attention is only called to the negative aspect of fermentation, that is the annoying gas formation in the intestine and the escaping of that gas as flatus.

However, the acids produced on fermentation stimulate peristaltis of the intestine, in this way counteracting constipation. And constipation in turn is the cause of many of the so-called civilization diseases.

So we must not forget the beneficial effect legumes have on constitution other then by their nutritive value.

SUMMARY

This paper first briefly reviews relevant investigations of research groups in the U.S.A. Most of those investigators are of the opinion that intestinal gas —in particular its hydrogen component— is formed by fermentation of the low molecular weight galactosido-oligosaccharides, raffinose and stachyose; these are not digested by the human digestive enzymes.

Our work calls attention to the content of all indigestible carbohydrates of beans; it accounts for about 15 to 20 per cent of the dry beans. The insoluble cell wall constituents cellulose, hemicellulose, and lignin constitute about 12 to 15 per cent, the remaining 5 per cent is soluble; it consists of pectin and the galactosido-oligosaccharides.

A simple in vitro method was developed to determine the insoluble indigestible residue of beans. When analyzing the faeces of human subjects while on a bean diet, only small percentages of the indigestible residue of the beans were found back in the stools. This part must have been fermented in the large intestine, resulting in the development of gas.

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The use of common beans as human food in Tanzania

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Tanzania lies on the East Coast of Africa with 937,062 Square kilometres and a population of 13.7 million made up of one twenty nine different tribes having a rich diversity of food habits and taboos. The tropical climate is highly variable from very dry to heavy equatorial types with a subsequent range of ecological zones suitable for tropical and subtropical crops.

The national malnutrition problem, especially protein calorie deficiency has been recognized since early this century and to date, several practical suggestions have made to effect remedial measures (1, 2). Surveys have indicated that 20-30% of the vulnerable groups especially the underfives are malnourished (3, 4). The main consequences include high infant mortality, low resistance to disease, poor early mental development, morbidity and low productivity among workers, hence little contribution to national development.

Among the potential solutions, the use of common beans and other legumes has been advocated as they are grown and eaten in all the ecological zones and are relatively cheap in urban centres selling at fluctuating prices of 60-90 cents (Tanzanian) per kg. The price is much lower in rural markets. The use of animal and milk products is limited to the tsetse free areas where livestock rearing is possible, elsewhere, they are too expensive. Steak for example retails at between $ 0.8 to $ 2.5 (U.S.) per kg especially in towns. The prices are prohibitive for a population whose per capita income averages about $ 90 (U.S.) per annum and about 80% of this is spent on food especially in towns.

From the table it is evident that Tanzania produces about seven million metric tons of food annually for about 13.7 million people. Cereals usually eaten with legumes account for 25% of total production. Legumes with oil seeds average 30% of all food produced. Legumes production is reasonable averaging about 180,000 tons

over the five years considered. They contribute substantially to our nutritional needs. However, production must increase to cope up with incerasing population, estimated at 380,000 people annually to augment the limited animal and milk products (see table). The increment must be sizeable to offset losses during storage estimated at 20-30%.

Crop culture research on common beans and other legumes has not received much attention except soya beans. Most types grown at the peasant level are mixed varieties with *Phaseolus vulgaris* species dominating. Limited work in Northern Tanzania was concentrated on varieties suitable for export, especially for canning (7, 8). Established varieties include: - For greens: - Top cross stringless, Tendergreen stringless, Brittlewax and Brown beauty. Dry shelled beans: - Brown Dutch and Canadian Wonder. For canning: - Tengeru varieties No. 8, 9, 15 and 16. These varieties originally from South America were selected and recommended for commercial production but due to fluctuations in the world market and unorganized production, the export business has not been a success and production has been mainly for local consumption.

However, recently increased production has been undertaken in the North for the production of common beans by the National Agricultural and Food Company (a parastatal organization) and the recently formed seed company will ensure the production of quality seed. The introduction of an incentive price of Shs. 0.85 for grade A beans and Shs. 0.65/Kg for mixed beans all over the country is likely to lead to increased acreage and higher production (9).

PREPARATION AND UTILIZATION:

Common beans (mainly *Phaseolus vulgaris*) are mostly grown for local consumption as green beans in the pod and dry shelled beans; for export to Europe by large scale growers and for canning especially haricot beans (baked beans), on a small scale.

Beans are usually eaten mixed with other food types or as a relish (side dish). Many recipes incorporating beans are used and they differ from tribe to tribe, but several standard recipes have been evolved from the traditional ones with modifications in the proportions towards affecting balanced diets (10, 11). They can be grouped as follows:
Beans (mixture or relish) with (a) cereals (maize, millets, sorghum and rice). (b) Root crops (sweet potatoes, cassava)

(c) Starchy fruits (bananas). Often vegetables and condiments are added.

The common method of preparation includes, washing the beans, soaking them over night, washing and cooking for up to 6 hours with the addition of sodium bicarbonate or common salt, till tender. Usually where a mixture of cereals or another starchy food (60% - 70%) is intended, the beans (40 - 30%) are half cooked, washed and the other food stuffs are then added and cooking completed. Whole maize takes longer to cook, so when it is used, the maize and the beans are partially cooked separately, mixed after washing, and finally cooked together and garnished.

When used as a relish (side dish), the beans are cooked as above, sometimes mashed, condiments added, further cooked and diluted to a thick gravy. At times, the beans are dehulled before mashing, and in both cases vegetables are added according to availability.

Stiff maize porridge and beans form the bulk of food for most institutions such as hospitals, schools and prisons, much like potato diets in the Western World.

The nutritional value of beans and other legumes is well documented including amino acid content and protein quality (12, 13) but investigations currently underway in the Ministry of Agriculture on local types might reveal highly nutritions varieties. The varieties of common beans now produced, average 22 - 25% protein with fair contribution of the B-group of vitamins and minerals. However, cooking causes considerable loss in the water soluble and heat labile vitamins especially thiamine (14). Cooking in the alkaline medium (bicarbonate of soda crude or refined), partly hydrolyses some of the protein while heat destroys the antidigestive and toxic factors and increases the net protein utilization value (15). A better amino acid balance is reached though contribution from the cereals (richer in methionine) where such mixtures are used, as it is established that the two amino acid patterns complement each other.

BABY WEANING FOOD PRODUCTION:

Traditionally, common bean dishes are usually meant for older children and adults. Mixtures are rarely used for infants especially the under fives except where they are weaned directly into adult diets. To combat malnutrition two serious attempts have recently been made. Kreysler (16) has reported studies in Lushoto district

PRODUCTION OF MAJOR FOOD CROPS IN TANZANIA
Unit - 1000 Metric Tons.

	1967	1968	1969	1970	1971
Cereals					
Maize	560	678	536	650	547 *
Millet and Sorghum	292	275	236	245	255 *
Rice (Paddy)	114	136	136	182	206 *
Wheat	31	44	39	61	63 *
Legumes					
Dry beans (mixed)	102	105	110	207	150
Dry Peas	6	5	13	14	14
Chick peas	4	20	11	10	12
Pigeon peas	10	12	11	10	11
Cowpeas	16	22	10	8	9
Root Crops					
Cassava	1200	1200	1300	1500	787 *
Sweet potatoes and yams	263	279	213	213	235 *
Potatoes	35	57	45	48	44 *
Fruit and Vegetables					
Vegetables (different types)	31	42	45	47	66 *
Onions	17	28	31	47	29 *
Bananas (less plantains)	440	363	521	679	514 *
Pineapple	32	33	35	36	-
Oranges and tangerines	23	19	65	146	320 *
Oil Seeds					
Groundnuts	62	65	38	38	29 *
Sesame	93	113	102	108	110
Soyabeans	-	.15	.11	.12	.16 *
Sunflower seed	11	8	12	6	4 *
Cotton seed	106	87	120	120	146 *
Coconut	290	270	300	321	-
Animal Products					
Meat from Indigenous Animals (beef, mutton, pork)	137	132	148	154	155
Poultry	227	218	242	253	-
Milk (cow and goat)	662	688	737	757	768
Hen eggs	133	136	141	144	150
Fish (estimated)	121	122	125	125	130 *

* Source - Ministry of Agriculture.
- Data not available.

on a cheap protein food trade named "MASUMA" containing two parts of maize and one of bean (*P. vulgaris*) flour and 20% sugar. The protein content was 13.1% without the sugar. A feeding study on eleven children over eighteen months resulted in reasonable nutritional improvement. Pilot scale production in the village by milling the ingredients together in a hammer mill, packaging with cooking instructions and retailing in one kilogram containers at Shs. 1.50 was initially successful.

The Ministry of Agriculture experimentally produced a product "named" "AFYA" containing 60% maize flour, 25% bean flour, 5% fish protein concentrate (FPC) and 10% sugar (17). The product with 16.6% protein content was acceptable to several taste panels and children under five coming to clinics. The product was intended to provide urban mothers with a cheap weaning food for their young children and to encourage rural mothers to prepare their own weaning food mixtures. Test marketing was carried out at 60 Tanzanian cents per 250 g in printed polyethylene bags. Initial trials were fairly successful in two urban centres.

Both "Masuma" and "Afya" production and test marketing attempts eventually failed because of lack of follow up and promotion. Since the maize and the bean flours were raw, the product had a limited shelf-life, mostly due to high moisture content (10-12%) and continued biological and biochemical activity. There was a risk of poor digestibility and biological utilization because of incomplete inactivation of antidigestive factors in the beans, when cooking was not thorough.

PILOT PLANT PRODUCTION OF INSTANT BEAN RELISH:

Kidney beans were soaked in water over night, washed, pressure cooked for 30 minutes at 15 p.s.i. and drained. Condiments were added (4% salt, 4% tomato puree and 2% curry powder). The mixture was mashed with a resultant solids content of 45%. The mash was drum dried on a Kestner Patent Laboratory drier. Optimum conditions were 40 p.s.i. steam pressure and 1.12 r.p.m. using an applicator roller. The flakes were hammer milled through a 0.6 mm. screen giving a golden brown nice flavour flour (4% moisture content) which reconstituted readily in warm water giving an instant relish which was highly acceptable to a taste panel (18).

Production of this type of relish could be undertaken as it has been found possible and economically feasible by other investigators

using beans, peas and lentils (19, 20). Large scale production would possibly considerably increase the use of instant relishes for eating with starchy preparations as traditionally done especially in urban areas, since the cooking of pulses is very laborius and time consuming.

PROSPECTS:

Production of common beans and other legumes must increase dramatically as a cheap means to close the protein calorie gap in the population which is increasing at 2.7% annually. Intermediate technology ought to play a big role, especially in the rural areas in village level processing to produce food mixtures for the nutritionally vulnerable groups. High Protein Product Development involving pulses as evident in current world wide research effort, is possibly the salvation of the Developing World.

SUMMARY

Malnutrition among vulnerable groups is minimized by the traditional use of beans usually eaten with starchy foods especially cereals. Baby weaner food mixtures containing maize, beans, fishmeal and sugar have been accepted. Village level production is feasible. Instant bean relish, produced on a drum drier, could be commercially produced.

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Weaning food prepared from whole soybeans and bananas by drum drying

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INTRODUCTION

The demand for protein continues to increase with accelerating world population and greater awareness of the importance of protein nutrition. In developing countries malnutrition is often endemic due to excessive dependence on low protein foods. Obtaining diets which contain adequate amounts of even the major nutrients, namely carbohydrates, protein and fat, is a serious problem in many areas. This problem is most serious in the diets of children since they require much higher levels of protein which is the most expensive nutrient. Thus, it is important to develop foods, especially weaning foods, which provide adequate levels of all major nutrients and which utilize local materials.

Since the soybean contains 20% oil and 40% protein, it is an excellent source both of energy and protein. The amino acid balance of soybean is one of the best which is readily available from plant sources. Thus, the soybean has great potential to people who must rely mostly upon vegetable sources for protein. Recent studies have shown that soybeans can be grown with high yields in many developing countries (2, 3). According to Mustakas et al (5). "The basic problems of upgrading the soybean to human food can be listed as follows: (a) inactivating growth inhibitors, (b) producing a bland and palatable product, and (c) obtaining good storage stability".

In most regions where malnutrition is a serious problem, bananas are grown abundantly. Bananas are eaten and well liked by children everywhere. They are high in carbohydrates and a

good source of certain minerals and vitamins, especially iron, vitamin A, riboflavin, niacin and ascorbic acid. However, there is often a considerable loss of bananas due to spoilage before the fruit can be distributed and consumed. This is mainly due to a relatively short holding period after harvest until the fruit is overripe.

A combination of soybeans and bananas should provide a good balance of nutrients. The protein and oil can be reduced to more desirable levels and the carbohydrate, mineral and vitamin levels increased. A banana flavored product would have widespread appeal to children and the storage characteristics of the mixture are stabilized by drying.

This report covers the development of a drum dried soybean-banana weaning food and an investigation of the parameters related to its flavor, color, texture, and storage stability. A preliminary nutritional evaluation was also carried out (7).

MATERIALS AND METHODS

All chemicals were reagent grade unless stated otherwise. Amsoy variety soybeans were used which contained about 6% moisture. Bananas of the Valery variety and "Cabana" brand bananas of unknown variety were purchased in local markets and held at room temperature until they ripened to the desired level.

Manufacture

The soybean-banana flakes were prepared according to the general method outlined in Figure 1. Experimental variations in the method are outlined later. Unless otherwise noted the weaning food was prepared using a 1:1 solids ratio of soybeans and bananas. All samples were drum dried on a Buflovak laboratory, 15 x 20 cm (6 x 8 in.) drum drier, model ALC-4. Drying was carried out at a steam pressure of 2.8 kg/cm^2 (40 psig), with a drum spacing of 0.025 mm (0.01 in.). Samples were dried to a moisture level of less than 4%.

Soaking and blanching

Samples of soybeans were soaked and/or blanched and used for the preparation of the weaning food.

Samples	Soaking Solution	Blanching Solution
A	none	tap water
B	none	0.5% $NaHCO_3$
C	tap water	tap water
D	tap water	0.5% $NaHCO_3$
E	0.5% $NaHCO_4$	—
F	0.7% H_3PO_4	0.5% $NaHCO_3$

Two hundred grams of soybeans were soaked for five hours in one liter of solution and blanched for 30 minutes in two liters of solution.

Banana ripeness — Bananas were held at room temperature and allowed to develop to four levels of ripeness which was indicated by skin color and percent soluble solids. The effect of ripeness on flavor, color, texture and off-flavor and storage stability of the weaning food was evaluated organoleptically.

Addition of SO_2 — The effect of adding 0 to 400 ppm of SO_2 (relative to dry soybean weight) was evaluated organoleptically with respect to flavor, off-flavor, color and stability to storage at 38°C in vacuum. Addition of 200 ppm SO_2 was used in all experiments except for the storage and addition of SO_2 studies.

Soybean: banana ratio — Soybean: banana flakes were prepared using soybean: banana ratios of 7:3, 6:4 and 5:5 on a dry solids basis. They were evaluated organoleptically with respect to flavor, off-flavor, texture and color.

Storage Stability

Preliminary storage tests were conducted for 13 months on samples prepared using Clark 63 variety soybeans. The dry beans were blanched in tap water containing 0.4% $NaHCO_3$. A 5:5 soybean: banana ratio was used and about 400 ppm SO_2 was added on a solids basis. Samples were stored at 38°C in air, at 38°C in vacuum. The two variables studied were SO_2 levels was stored ate —40°C was used as a reference.

Additional storage studies were carried out for 2 months at a 38°C in vacuum. The two variables studies were SO_2 levels and banana ripeness. Sulfur dioxide levels were 0, 200, 300 and 400 ppm. The weaning food was prepared using bananas containing 21.5% (unripe) and 24.0% (ripe) soluble solids. All samples were vacuum sealed in fruit enamel cans for storage. The samples were evaluated organoleptically after 0, 1 and 2 months.

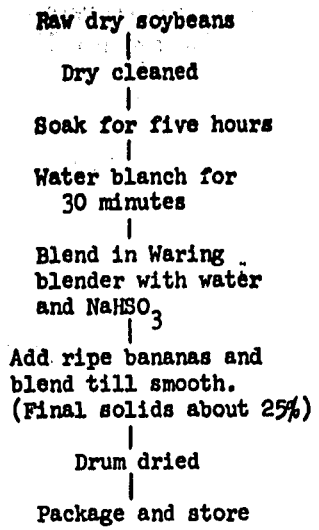
Raw dry soybeans
Dry cleaned
Soak for five hours
Water blanch for 30 minutes
Blend in Waring blender with water and $NaHSO_3$
Add ripe bananas and blend till smooth. (Final solids about 25%)
Drum dried
Package and store

Figure 1. Preparation of Soybean-banana Weaning Food

Chemical Analysis

Residual SO_2 in the dehydrated soybean-banana flakes was analyzed by the direct titration method described by Potter and Hendel (6). Moisture was determined by measuring weight loss of samples dried in a vacuum oven at 60°C for 24 hours. Nitrogen was determined by the micro-Kjeldahl method as described in AOAC methods, (1). The percent nitrogen was multiplied by 6.25 to give percent protein (4).

Soluble sugars were estimated by extraction with ethanol, and analysis for reducing and non-reducing sugars as follows:

Weighed samples (about 0.2 g) were refluxed overnight with 50 ml of 80% ethanol. The ethanol mixture was quantitatively removed and filtered through Whatman Nº 1 filter paper. The volume of the filtrate was reduced to about 40 ml by evaporation on a steam bath after which it was diluted to 100 ml in a volumentric flask. A 50 ml aliquot was analyzed for reducing sugars using the Munsen-Walker general method for reducing sugars (1). Five ml of concentrated HCl was added to the 50 ml remaining in

the volumetric flask and it was allowed to stand overnight at room temperature in order to hydrolyse non-reducing sugars. The sample was neutralized with 25% Na OH and analyzed for reducing sugars as described above (1). The difference in amounts of reducing sugar before and after acid hydrolysis was considered to be non-reducing sugars. The value obtained after acid hydrolysis was considered to be total soluble sugars.

Starch was determined on the residue remaining after ethanol extraction using a modified AOAC method (1). The residue was quantitatively transferred to a flask and water added to give a volume of about 50 mls. Five ml of concentrated HCl was added and the mixture refluxed overnight. The mixture was nearly neutralized with 25% NaOH, diluted to 100 ml and the reducing sugar determined by the Munsen-Walker method described above.

Total solids of the bananas were routinely determined by using a refractometer and adding one percent to the value obtained. This value corresponded well to the value obtained by measuring weight loss of ripe bananas in a vacuum oven.

Oil was determined on a dry, ground and weighed sample by extraction for six hours with petroleum ether (Skelly F) in a Soxhlet apparatus. After extraction, the solvent was evaporated, the oil was dried at 60°C in a vacuum oven for 24 hours and weighed.

Organoleptic Evaluation

Flavor was judged by the presence of a pleasing banana flavor. The off-flavor score was determined by the absence of "beany" or other off-flavors. Good texture was considered to be grainy and not pasty. A light golden color was considered desirable. The panel consisted of ten staff members with experience gained in judging this product during the preliminary studies.

Samples were presented dry and after rehydrating 12 g of sample with 33.3 ml of water.

Triangle tests were made for color of the dry flakes and for flavor, off-flavor ,texture and color of the rehydrated product. Tests for flavor, off-flavor and texture were made under a red light to prevent bias due to color. Tests for color were made under a simulated daylight lamp.

Samples were also evaluated using a hedonic scale from 1-9 in which 9 represented an excellent score, 5 barely acceptable and 1 very poor. Six samples were presented at each evaluation, and each was rated for the organoleptic qualities described above.

Statistical Analysis of Organoleptic Results

The organoleptic data was arranged in a randomized complete block design. An analysis of variance was used for each set of data. Within each evaluation, Duncan's New Multiple Range test was applied to determine whether or not there was a significant difference among the treatment means (8).

Measurement of the Bacterial and Mold Populations

Total plate counts were made in duplicate using TGE agar (Difco). Plates were incubated at 37°C for 48 hours before counting. Mold counts were made in duplicate using Potato Dextrose Agar (Difco). These plates were incubated at 32°C for 48 hours and then counted.

RESULTS AND DISCUSSION

Soybean Treatment — Several variations of soaking and blanching were investigated as indicated in Table 1 and finished products were tested organoleptically for flavor, off-flavor, texture and color. The best flavor was obtained by soaking and blanching in 0.5% $NaHCO_3$ solution and the least desirable by soaking and blanching in tap water. Mean hedonic flavor scores ranged from 7.6 to 6.8 and differences were not great. Differences in off-flavor and texture ratings were also small, the range of the mean score being 0.7 and 0.4, respectively. However, statistical analyses, using Duncan's New Multiple Range test showed that soaking and blanching treatments were generally significantly different and that treatments using $NaHCO_3$ were superior to those employing tap water.

Color was adversely affected when $NaHCO_3$ solutions were used for soaking and/or blanching. Use of $NaHCO_3$ in the soaking and blanching solutions resulted in the darkest color while use of tap water produced the lightest color in the finished product, Table 1. Statistical analysis revealed that color of both the dehydrated and rehydrated finished products was significantly improved when $NaHCO_3$ was not added to soak and blanch solutions.

It is also interesting that the range of the color scores was substantially greater than noted for flavor, off-flavor and texture. Triangle tests were also performed on the samples and analysis of the data confirmed the findings obtained when the products were rated using hedonic scoring. The data indicate that the additional cost for equipment, time and labor to soak the beans prior to blanching does not appear to be justified. Use of $NaHCO_3$

to improve the flavor may not be desirable because of the adverse effect on color.

TABLE 1
EFFECT OF DIFFERENT SOAKING AND BLANCHING SOLUTIONS ON ORGANOLEPTIC SCORES OF SOYBEAN-BANANA FLAKES

Sample	A	B	C	D	E	F
Soaking Solution	none	none	tap water	tap water	0.5% $NaHCO_3$	0.7% H_3PO_4
Blanching Solution	tap water	0.5% $NaHCO_3$	tap water	0.5% $NaHCO_3$	0.5% $NaHCO_3$	0.5% $NaHCO_3$
Organoleptic Scores for:						
Flavor	7.2	7.2	6.8	7.4	7.6	7.2
Off-flavor	7.6	7.8	7.4	7.8	7.9	7.2
Texture	7.2	7.5	7.1	7.5	7.4	7.5
Dry color	8.1	7.1	8.7	6.7	6.8	6.7
Rehydrated color	8.0	6.2	8.6	6.7	7.1	6.5
Duncan's New* Multiple Range Test for:						
Flavor	E D A B F C					
Off-flavor	E B D A C F					
Texture	D F B E A C					
Dry color	C A B E F D					
Rehydrated color	C A E F D B					

*Significant at the 5% level.

Banana Ripeness — The typical appearance of bananas having the four levels of ripeness that were used in this experiment was based on colour. The soluble solids contents of the bananas, which ranged from green to ripe, were 21, 23.2, 24 and 25%. These are labelled A, B, C and D, respectively.

The triangle test was employed for comparison and evaluation of samples. Significant flavor differences were not found between some samples when there were small differences in soluble banana solids. However, when differences in banana solids were larger, a highly significant difference was found and preference was always shown for samples prepared from bananas with higher soluble solids.

There were no significant differences between samples for off-flavor in the banana ripeness study. Some significant texture differences were found between certain samples. Products prepared with green fruit which was lower in soluble solids were invariably more pasty and less preferred when compared to samples prepared with the higher soluble solids bananas. This phenomenon can be explained by the high starch content in the greener or less ripe bananas.

The triangle test showed highly significant color differences between samples containing the riper or higher soluble solids bananas as compared to samples of lower soluble solids, Table 2. The very favorable effect of the higher soluble banana solids on color was clearly demonstrated and should influence further research and acceptability studies.

TABLE 2

EFFECT OF BANANA RIPENESS ON COLOR AND FLAVOR DIFFERENCES OF WEANING FOOD - TRIANGLE TESTS

Samples Compared	A and B		C and D		B and D		E and F	
Flavor								
Correct Judgments/Total	12/20		8/20		11/20		15/20	
Significance Level for Choice of Correct Sample	5%		n.s.		5%		0.1%	
Preferred Sample	B		---		D		B	
Significance Level for Preference	2.5%		---		0.5%		0.5%	
Color	Dehyd	Rehyd	Dehyd	Rehyd	Dehyd	Rehyd	Dehyd	Rehyd
Correct Judgments/Total	9/20	18/20	13/20	13/20	20/20	20/20	20/20	20/20
Significance Level for Choice of Correct Sample	n.s.	0.1%	1%	1%	0.1%	0.1%	0.1%	0.1%
Preferred Sample	---	B	D	D	D	D	B	B
Significance Level for Preference	---	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%

A - prepared with bananas of 21% solids
B - prepared with bananas of 23.2% solids
C - prepared with bananas of 24% solids
D - prepared with bananas of 25% solids
E - prepared with bananas of 23% solids
F - prepared with bananas of 26% solids

Dehyd - Dehydrated color
Rehyd - Rehydrated color
n.s. - not significant

Results of this study do not explain why less ripe banana produce a weaning food of inferior color. However, the grey color may be due to presence of free tannins and the formation of dark iron-tannin complexes. The amount of tannins decrease as bananas ripen (9). It is also possible that some of the greener fruit contains small amounts of chlorophyll which would adversely affect color.

Soybean-Banana Ratio

Table 3 defines the various soybean-banana ratios used and present the hedonic scores for the organoleptic evaluation of the products and results of the Duncan's New Multiple Range test.

TABLE 3
EFFECT OF SOYBEAN: BANANA RATIO ON ORGANOLEPTIC SCORES OF SOYBEAN: BANANA FLAKES

Sample	A	B	C
Soybean:Banana Ratio	7:3	6:4	5:5
Organoleptic Scores for:			
Flavor	6.4	7.3	8.4
Off-flavor	7.5	7.9	8.2
Texture	7.0	7.7	7.8
Dry color	8.2	8.6	7.5
Rehydrated color	8.2	8.2	5.4
Duncan's New* Multiple Range Test for:			
Flavor	C B A		
Off-flavor	C B A		
Texture	C B A		
Dry color	B A C		
Rehydrated color	A B C		

*Significant at the 5% level

All of the samples were prepared with bananas of 26.2% soluble solids. Examination of the data in Table 3 shows that as the soybean-banana ratio is varied from 70:30 to 50:50 that hedonic flavor scores increase from 6.4 to 8.4. The results of the Duncan's New Multiple Range test for flavor show there is a significant difference between all samples. Quite obviously, as the level of banana solids is increased in the finished product, the weaning

food is sweeter and contains more banana flavor. However, it should be noted that scores for all of the samples were sufficiently high for the flavor to be considered acceptable. It should also be considered that feeding tests with children may show that samples containing the higher soybean solids are quite acceptable.

It is interesting to note, Table 3, that off-flavor and texture values and the statistical analyses generally paralleled the results for the flavor evaluations. It is possible that off-flavor and texture values are influenced by the flavor However, all samples were rated quite acceptable for off-flavor and texture The results of the statistical evaluation on dehydrated and rehydrated color is shown in Table 3. These results clearly show that samples containing increased percentages of soybean were rated higher for both dehydrated and rehydrated color. When whole soybeans are prepared and drum dried without any additive they are light and bright in color and the effect of increasing soybeans in the mixture is very apparent when direct comparisons can be made.

This study showed that increasing soybeans and reducing the banana level with weaning food will adversely affect flavor and improve color. Further work is required before the question of optimum soybean-banana ratio can be clarified. This must be considered on the basis of product acceptability for the child and on the nutritional value and need for the product.

Storage Studies

Two storage tests were conducted on the soybean-banana weaning food. The first study was started when the work outlined in this report was first underway. The procedures used in preparing these samples have been described in the Methods section and they were consistent with the technology which was known at that time. Table 4 presents the hedonic means for organoleptic evaluations as well as the data on the Duncan's New Multiple Range test for samples stored 13 months. This study is very important because it shows that the soybean-banana products exhibit remarkable stability during storage. Even the air packed sample stored at 38°C had acceptable flavor, off-flavor, texture and color in the dehydrated and rehydrated forms after 13 months. Both samples A and B, Table 4, were of very good flavor, off-flavor, texture and color in the dehydrated and rehydrated forms. A comparison of air versus vacuum packs stored at 38°C, samples B and C, Table 4, show that vacuum packing is of real value if the product is to be subjected to long term storage at high temperature.

TABLE 4
ORGANOLEPTIC RESULTS FOR THE 13 MONTH STORAGE STUDY

Sample	A	B	C
Hedonic Mean for:			
Flavor	7.40	7.70	6.20
Off-flavor	7.50	7.80	5.70
Texture	7.90	7.70	7.70
Dehydrated color	7.90	7.30	7.10
Rehydrated color	7.80	7.10	6.30
Duncan's New* Multiple Range Test for:			
Flavor	B A C		
Off-flavor	B A C		
Texture	A B C		
Dehydrated color	A B C		
Rehydrated color	A B C		

*Significant at the 5% level. A - air packed, stored at 70°F. B - vacuum packed, stored at 100°F. C - air packed, stored at 100°F.

Table 5 presents the hedonic means and results of Duncan's New Multiple Range test for flavor, off-flavor and texture of samples subjected to 38°C storage for determination of the optimum SO_2 level. Examination of the data on flavor shows that certain samples are statistically superior. However, no predictable pattern was apparent during the two month storage. Further examination of Table 5 indicates that off-flavor and texture change, although often different, follow no apparent pattern over the tow month storage at 38°C. Thus, no conclusion or predictions can be drawn from the analyses of the flavor, off-flavor and texture data. However, it seems reasonable to believe that if storage time was increased the favorable value of SO_2 would become apparent.

The hedonic means and result of Duncan's New Multiple Range test for dehydrated and rehydrated color are shown in Table 5.

TABLE 5

EFFECT OF SO_2 LEVEL ON ORGANOLEPTIC QUALITY OF SOYBEAN: BANANA (1:1) FLAKES

Storage Time	0				1 month				2 months			
Treatment	A[a]	B	C	D	A	B	C	D	A	B	C	D
Hedonic Means for:												
Flavor	7.80	7.55	7.80	7.35	7.55	7.45	7.75	7.50	7.50	7.75	7.35	7.45
Off-flavor	7.85	7.95	8.25	8.10	7.75	7.80	8.20	7.85	7.50	7.40	7.85	7.80
Texture	7.70	7.70	7.90	7.65	7.60	7.80	7.85	7.85	8.00	7.70	7.60	7.75
Dehydrated color	7.55	7.35	7.75	8.30	6.80	7.10	7.65	8.30	7.30	7.60	7.55	8.25
Rehydrated color	6.00	6.95	7.15	8.00	5.70	6.90	7.10	8.05	5.95	6.85	7.45	8.85
Duncan's New* Multiple Range Test for:												
Flavor	A	C	B	D	C	A	D	B	B	A	D	C
Off-flavor	C	D	B	A	C	A	B	D	C	D	A	B
Texture	C	A	B	D	D	C	B	A	A	D	B	C
Dehydrated color	D	C	A	B	D	C	B	A	D	B	C	A
Rehydrated color	D	C	B	A	D	C	B	A	D	C	B	A

*Significance at the 5% level.
[a]A - 0 ppm SO_2 added. B - 200 ppm SO_2 added. C - 300 ppm SO_2 added. D - 400 ppm SO_2 added.

There were significant differences in mean color values and generally the color was improved as SO_2 levels in the product were increased. The favorable effect of increased SO_2 level was more apparent in the rehydrated color values and this effect was enhanced as storage time was increased to two months. It can be predicted that with increase in storage time the protective effect of SO_2 on product color would be more pronounced.

In another two month storage experiment the effect of banana ripeness was investigated. Soybean-banana weaning foods were prepared using bananas with 21.5% and 24% soluble solids and these samples were stored for 0, 1 and 2 months at 38°C. It is interesting that the weaning food prepared from ripe bananas, 24% soluble solids, was very significantly superior with respect to all organoleptic characteristics. Organoleptic rating for flavor, off-flavor, texture, dehydrated and rehydrated color were always substantially superior for the samples prepared with high soluble solids bananas. Thus, this experiment confirms the importance of using ripe bananas of high soluble solids in the preparation of the soybean-banana weaning food.

Nutritional Evaluation — The results of chemical and microbiological analysis are shown in Table 6. There is a good balance of major nutrients. In addition, calculations based on composition tables for soybeans and bananas suggest that this weaning food may be a good source of iron, thiamine and riboflavin. However, the weaning food has not been analyzed for minor nutrients at this time.

TABLE 6
CHEMICAL AND MICROBIOLOGICAL ANALYSES OF A 1:1 SOYBEAN-BANANA (SOLIDS RATIO) WEANING FOOD

Chemical Analysis	Percent by Weight as is basis	dry basis
Moisture content	2.87	---
Oil content	8.55	8.80
Protein content (N x 6.25)	22.34	23.00
Starch content	9.59	9.87
Total sugar	38.96	40.01
Microbiological Analysis	**Counts Per Gram**	
Total Plate Count	30	
Mold Count	0	
Total Plate Count on 13 Month Storage Sample	50	
Mold Count on 13 Month Storage Sample	0	

Nutritional studies which included this soybean-banana weaning food were published earlier (7). The amino acid analyses performed during these studies indicated that the soybean-banana was too low in methionine to give good PER'S with weanling rats. Supplementation of the weaning food with 0.5% methionine gave a PER of 2.2 (casein was 2.5). However, a diet which contained only the weaning food (and the vitamin and mineral supplement normally used) appeared to produce excellent growth of weanling rats. The average weight gain of 21 day old Sprague Dawley male rats was 69 g in 14 days. When the weaning food was supplemented with 0.5% methionine the average weight gain was 75 g. Corresponding weight gains averaged 48 g for rats fed a

diet which contained 10% casein protein. Since cereals such as corn and rice are normally good sources of methionine, the shortage of methionine in the weaning food probably is not serious for humans provided the diet is varied. The weaning food should be supplemented with 0.5 to 1% methionine if it is the major or only source of protein in the diet. Further studies are planned to correct this problem by adding a third ingredient which is higher in methionine.

Microbiological Evaluation — Total plate counts and mold counts were done on the weaning food before and after long term storage. It is clear that the microbiological quality is very high. If the dry product is stored in sealed containers there should be no problem with micro-organisms even in tropical countries.

Conclusions

Soybeans are a potential source of good quality protein and they are becoming more available throughout the world. However, people will resist using soybeans for food unless it can be processed into a product with highly acceptable flavor. It should also be easily identified with locally acceptable foods and contain a proper balance of major nutrients.

Experimental studies on drum dried soybean-banana weaning food were made and products were evaluated for organoleptic and storage properties. It was shown that use of very ripe bananas (24% soluble solids) was necessary for the preparation of a high quality weaning food. Addition of $NaHCO_3$ in the soybean blanch water resulted in better flavor but poorer color in the finished product. The use of $NaHCO_3$ for soaking and/or blanching is probably not justified. If used, a balance between improved flavor and poorer color must be found. Soaking the soybeans prior to blanching did not result in sufficient improvement in the weaning food to justify the added expense. A soybean: banana ratio between 3:2 and 1:1 resulted in the best overall organoleptic characteristics but the precise ratio was not defined in these studies. Addition of 300-400 ppm of SO_2 is recommended to lighten the color and improve the color stability during long term storage. The weaning food had excellent stability during storage at high temperature. For extended storage in the tropics the weaning food should be stored under vacuum.

In summary, the best soybean-banana weaning food was prepared in these studies by blanching the soybeans for 30 minutes in tap water, grinding the beans with tap water, adding 400 ppm SO_2,

blending ripe bananas into the soybean slurry in a ratio of 1:1 or 3:2 (soybean: banana solids) and drum drying to a moisture content of less than 4%.

Further studies are necessary to improve the amino acid balance. A more complete investigation of the effect of banana ripeness and composition must be carried out. Further refinement of processing conditions is needed. Materials such as citric acid and ascorbic acid should be tested to determine if they will improve the color of the final product. More complete nutritional studies are needed as well as extensive acceptability investigations with children. If continued studies are promising, it will be necessary to investigate production of the soybean-banana weaning food on a larger scale to determine economics of the process.

SUMMARY

Good tasting, high protein weaning foods were prepared by drum drying soybean-banana slurries. The typical "beany" or "painty" flavor of soybeans was completely prevented by water blanching the whole soybean. The blanched beans were finely ground and blended with the bananas before drying. Optimum ratios of soybean: banana were 1:1 and 3:2 on a solids basis. Most desirable flavor, color and storage stability was obtained when fully ripe bananas having at least 24% soluble solids were used. Addition of $NaHSO_3$ improved the color and flavor stability during storage. The weaning food had very good stability in storage for 13 months at 38°C under vacum and at 21°C in air. The 1:1 soybean: banana mixture contained 23% protein, 8.8% oil and 50% carbohydrates and when rats were allowed free access to the weaning food they appeared to have normal growth. It is suggested that soybean: banana flakes would be acceptable as high protein weaning foods with little or no modification to suit local preferences.

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The vital role of legumes in human nutrition

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Leguminous seeds are widely grown and utilized for food in many parts of the world. Depending on the climatic conditions of the area, certain types have become more dominant than others. In West Africa, legumes usually grown are Cowpeas *(Vigna unguiculata)*, Groundnuts *(Arachis hypogea)*, Lima beans *(Phaseolus lunatus)*, Bambara groundnuts *(Voandzea subterranea)*. Others are Pigeon peas *(Cajanus cajan)*, Sword bean *(Canavalia sp.)*, Locust bean *(Parkia sp.)* and Melon seeds *(Citrullus sp.)*.

Nutrient Contribution

These leguminous seeds used in West Africa, in common with all legumes, are important sources of nutrients (1). They are rich sources of proteins; the crude protein content of these dry legumes ranging from 18% to 30%. The legume proteins are of moderate quality, with N.P.U. between 40% and 55%. Thus the use of these legumes makes a valuable contribution to the protein values of diets based predominantly on root crops and cereal staples.

Most of these legumes contain small amounts of fats, about 2% on the average. Groundnuts, melon seeds (egusi) contain high quantities of fats in the range of 18% to 48%. Legumes contribute fair amounts of the minerals calcium and iron to the diet. Locust bean contains about 300 mg of calcium per 100 g compared with 20 mg in cowpeas.

Legumes provide variable quantities of most vitamins, depending upon their stage of maturity and state of dryness. Carotene, thiamine, riboflavin, niacin and ascorbic acid are present in many legumes. Groundnuts are very rich in niacin, containing about 15 mg per 100 g.

Preparation and Usage

In West Africa until recently, legumes are mainly home processed for consumption. Industrial canning of some legumes such as bambara and cowpeas has been initiated. In the home, the dried shelled seeds are soaked in water for about 12 to 24 hours. The soaking water is poured off, fresh water added and then boiled. The soaking renders the bean soft and the boiling invariably destroys most of the toxic substances present in the bean. Bean powder and bean doughs are also made. Groundnuts are usually roasted and milled into paste.

Legumes feature prominently in the diet of the areas where they are grown. In Ghana, cowpeas, bambara groundnuts, broad beans and groundnuts are used by the people in the Volta Region, Northern and Upper Regions and the coastal belt of the country. Various dishes and snacks are prepared with legumes. Bambara groundnuts and cowpeas are used in making stews and porridges which are eaten with plantain *(Musa paradisiaca)*, either fried or roasted. Cowpeas and rice are boiled together and a relish of pepper, onion and tomatoes fried together in palm kernel oil is added. This is a favorite dish for adults and children. Boiled groundnuts mixed with boiled maize are also eaten. Roasted groundnuts are used as snacks either alone or mixed with roasted maize. Roasted and milled groundnuts paste is used in making delicious soups and stews.

Cowpeas are soaked in water and ground into a flour. Water is added to the flour to make a dough and spoonfuls of this are fried in oil to yield bean cakes. The bean cakes are eaten with maize porridge or alone as snacks.

Protein-Energy Malnutrition

It is thus evident that legumes which are protein rich foods are widely used in diets in Ghana. Ironically, Kwashiorkor was first described from Ghana (then the Gold Coast) in 1931 and still the evidence of this nutritional deficiency disease, caused by lack of protein and excess energy in the diet of the child, has not abated. Apart from its toll on the child population in most developing countries and thus loss of the manpower resources, the detrimental effects of protein-energy malnutrition on the brain of affected children are now coming to light.

The child with kwashiorkor is clearly one little problem compared with the large number of children in the peri-urban areas,

the slums of the urban areas and the rural areas who are moderately but chronically malnourished. The small stature, the poor muscular development, the reddening of the hair and the relative lack of vigor are evidences of the poor nutritional status of such children.

The ecology of this malnutrition is very complex; but apart from the low economic level of the families of such children, the types of foods used in the diets of these families show clear evidence of their nutritional problems. For example, most infants in Ghana are breastfed for more than twelve months, but during the second six monts of the infant's life, various traditional weaning foods are introduced into the infant's diet. These traditionally do not include animal protein-rich foods or plant protein-rich foods such as legumes. These weaning foods are mainly gruels and porridges made from the cereals grown in the particular region. Maize, millet and sorghum porridges are extensively used in Ghana for infant feeding. The Net Protein Utilization (N.P.U.) of the sorghum gruel is 50% with a Net Dietary Protein Calories Percent of 4.5; the N.P.U. of the maize porridge is 45% with a NDpCal % of 3.8 (2).

Though infants receiving these weaning foods are also fed on the breast, these values of NDpCal are lower than those needed for good growth of infants. Those infants who manage to weather this storm are subsequently fed the adult diets. Though animal protein-rich foods, such as meat, fish, snails, and eggs feature in the adult diets, children normally get very little of this. Thus most diets of children are not high in protein values; the NDpCal of children's diets range from 3.2% to 4.1% (3, 4).

Paucity of Research on Local Legumes

Evidently, since animal protein foods are expensive and they are invariably reserved for the breadwinner, who is the male adult, a different approach to providing protein-rich foods for infants and children in developing countries has to be sought. Thus legumes which are protein-rich foods grown in most areas where protein-energy malnutrition occurs must be the foodstuffs of choice.

Mothers in Ghana do not normally feed legumes to infants because of two main reasons, 1) legumes tend to give indigestion in children and 2) the proper cooking of legumes is very tedious. In relation to these, it is surprising how comparatively little work has been done to improve the home processing, and develop industrial methods to process legumes, such as cowpeas, broad

beans, pigeon peas, bambara groundnuts, lima beans etc. commonly used in sub-tropical and tropical areas. A tremendous amount of work has been done with the soya bean, from which soya milk, soya flour, textured protein foods of every flavor and kind have been produced. Surely some of the soya bean processing techniques could be modified for other tropical legumes!

Legume Foods for Infants and Children

Because of the need to provide relatively cheap protein-rich foods for infants and toddlers, and since it is necessary to use foods already familiar to the people and in good supply, the legumes used as food in Ghana were investigated. The N.P.U. of cowpeas and rice mixture, in equal quantities was determined; a traditional food mixture of cowpeas with gari (grated dried cassava) was also prepared and the N.P.U. determined (1).

TABLE 1

Net Protein Utilization (N.P.U.) and Net Protein Calories Percent of Plant Protein Food Mixtures

Food Mixture	Protein Cal. %	N P.U.(op) %	NDpCal %
Rice and cowpeas	15.1	55	8.3
Gari and cowpeas	14.7	46	6.8
Maize and cowpeas	17.2	56	8.7

Other legume-cereal mixtures have been found to give high protein values. For instance, a mixture of 60% maize and 40% cowpeas was compared in its growth promoting effects with the traditional maize porridge given to infants, and also with two proprietary infant milk foods. It was found that the milk mixtures gave better growth performances, but the maize-cowpea mixture gave growth performance equal to 75% of that of the proprietary milk mixtures. The growth performance of the rats fed on the traditional maize porridge was only 21% of that of the proprietary milk mixtures.

TABLE 2

Effect of Feeding Different Foods on Weight Gain in Rats

Food Mixture	% Weight Gain	Days on Experiment
Proprietary Milk A	129.0	45
Proprietary Milk B	120.0	45
Maize	25.0	45
Maize and Cowpeas	90.1	45

Further comparisons of the growth performance of rats fed the legume-cereal mixtures compared with proprietary cereal-milk infant foods have shown the legume-cereal mixtures to be superior. Some of these legume-cereal mixtures were made by both traditional and modern processing methods. In the modern method, the legumes and cereals were ground into flour, then mixed in a kettle with boiling water, and the resultant paste passed over a double drum drier. Flakes of the mixture obtained were used as such or ground into powder form. The acceptability of these products among adults was very good. This is very important, because it has been found the mothers will not feed their children food mixtures which are not acceptable to them. Various traditional methods of preparing these mixtures are being studied in order to find suitable ones for village-level production of these food mixtures.

Infant Weaning Legume Foods

Efforts have been directed to find a plant protein-rich food which will be accepted by others for use in feeding their infants. Maize porridge which is used in Ghana during weaning has a white appearance and thus there is a semblance of milk. The legume bambara groundnut has been found to satisfy the color and texture prerequisites (5). Bambara groundnuts are subjected to boiling water grinding in a hammer mill, and the resultant slurry filtered. A white milk liquid is obtained which is boiled for about one hour. A white porridge is obtained which has a bland taste with a semblance of the traditional maize porridge. This product contains 17% protein compared with 9% protein of the maize porridge. Growth of rats on this has been good. A similar product has also been obtained from white cowpeas. These two products have been called NUTRIBAM and NUTRIPEA respectively.

TABLE 3

Effect of Feeding Different Foods on Weight Gain in Rats

Food Mixture	% Gain in Weight	Days on Experiment
Maize	28.1	40
Bambara Groundnut	76.8	40

It thus seems possible to make suitable food mixtures using various legumes as protein-rich foods. It is imperative that for the success of nutrition education programs concerned with infant and child feeding in developing countries, various legumes which already form the basis of the traditional diets be investigated and suitable products developed which should be recommended for use. The legume food mixtures developed should be easy to prepare, so that mothers can make these in the home. This is an overriding consideration which is forgotten when protein-rich foods are developed. Most of these are processed on the industrial scale and packaged for sale at prices which families earning $ 200 or less per annum cannot afford to buy. Most of these packaged foods make some impact at the beginning of their introduction, but soon sales go down and interest in the food is lost (6).

Production, Storage and Encouragement of Legume Consumption

A vital consideration which is not realised is the fact that the importance placed on animal protein-rich foods in nutrition education has adversely affected the production of legumes in some developing countries. The prices of these legumes have therefore risen and consequently there has been some reduction in consumption of legumes. The storage of legumes in various countries, but particularly in Africa, leaves much to be desired. Beans and cowpeas are always infested with weevils which bore into the grain and eat up the major part of it. Proper storage methods will therefore be of great value in legume preservation. There is therefore the urgent need for planned production of legumes which must be given priority in areas where animal protein is scarce. To encourage the use of legumes, storage facilities must be provided. There must be intensive research on production of legume food mixtures within the purchasing power of the people in developing areas of the world.

SUMMARY

Various legumes are grown and utilized in most tropical and subtropical parts of the world. These legumes are rich in protein and other nutrients, and thus make important contributions to the diets of people consuming them. Protein-energy malnutrition is also prevalent in these same areas. Very few legume foods and products are used in infant and child feeding in these areas. There is thus the need for more concentrated research on local legumes for use in making acceptable foods for children. The great amount of work done on soya bean products should provide the incentive for work with other legumes. Some legume-cereal products have been developed in Ghana which promise to be useful. Other legume weaning foods have also been developed. Simultaneously

with research work on legume product development, there is need for stimulation of legume production, proper preservation and storage of legumes, nutrition education of mothers in using legumes and their products in infant and child feeding.

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Perspectives on the consumption and utilization of legume seeds as food and feed

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Legume seeds are members of large family, including several species, and are known to survive in very different soils and climates. They have been part of the human diet for centuries and we could say that there must be very few people in the world today to whose diet legume seeds will not make some contribution.

From the nutritional point of view legume seeds are known to have a high protein content, to provide as many calories as cereals, and to be a reasonably good source of some B complex vitamins.

It is mainly because of their high protein content that legume seeds have been looked upon as one of the most promising suppliers of the protein need of the world.

It is also interesting to note that in spite of this potential the importance and contribution of legume seeds as a food source to meet protein needs has been minimal.

It has been shown that the present sources of protein in the human diet include 1/3 from animal sources and 2/3 from vegetable sources. In the vegetable sources the main contribution comes from cereals and the legume seeds supply 8 to 10% of the total (1). But it has been pointed out that these worldwide statistics are misleading as to the importance of legume seeds, because they are if not the only source of protein, at least the main source of it for about 50% of the rural population living in developing countries in the tropical belt (1).

It has been shown that in a few areas of Africa and Asia there is a very high consumption of beans, reaching figures of 400 g a day (2). On the other hand, in Latin America, bean consumption was said to reach its highest at an average of 60 to 70 g a day (3). This will supply, for some people, 30% of their total protein intake.

It is unfortunate that legume seeds have the connotation of poor man's food or poor man's meat, and this has caused and will cause social prejudices against their use.

Intake of legume seeds is said to decrease with higher income in affluent societies, and this seems to be true in some developing countries of Latin America. In India, and probably in other countries of Asia, there is a tendency to increase consumption of legumes in the higher, rather than in the lower, income groups (2).

Most of the legume seeds consumed today by humans are home cooked, and there has been very little industrial processing available. It is known that they are a difficult food to cook: time —and fuel— consuming in their preparation. It is also known that heating will increase digestibility and destroy toxic factors. But it has also been shown that temperature and time of heating are quite important factors, and inadequate home cooking will not destroy all the toxic factors (4).

It is interesting to note that legume seeds have been considered a "heavy" food, and their utilization by infants and small children has been restricted. This is certainly the situation in Brazil where only well strained beans and bean juice are offered to small children and infants. It was also shown in India that legume preparations, although of nutritionally high quality, were not suitable for children under 2 years because the volume to be eaten was too large to be digested (2). On the other hand it has been our experience and that of other authors that well-prepared soy and common beans are reasonably good sources of protein for both normal and malnourished children (5, 6).

The most important aspect of legumes in human feeding seems to be their potential to balance cereal-based diets. Cereal proteins are in general deficient in lysine and threonine, and legume seeds are good sources of these amino acids. Mixtures of cereal and legumes have been shown to have a higher protein value than their individual componentes, and this was shown in different laboratories and investigations (7, 8). Better utilization of the legume proteins can also be achieved by the supplementation of

their limiting amino acids, in general methionine (9). This approach has been shown to significantly enhance their protein nutritive value.

Considering all these facts it seems important to inquire about the future perspectives of legume seeds. It looks as though legume seeds have been rediscovered as potential source of protein for human feeding, and new and brighter prospects are now open.

What concerns about some of this enthusiasm is the lack of a more broad and coordinated effort to deal with the problem. Some people in agriculture think that higher yields and better agricultural practices are the answer; others stress better acceptability and/or better nutritive value of the legume seeds. Where to set priorities, which efforts to stress, and where to allocate funds are some of the important points. If we expect legume seeds to really make a contribution to fill the protein gap we have to look at their food chain. By this I mean to look at aspects of production, marketing, industrialization, and consumption, and to work simultaneously in several of them. I also mean that it is necessary to concentrate efforts in some of the more neglected areas. It seems, for example, that a lot of emphasis will be placed on legume agriculture and very little on legume storage or food technology, fields in which the gap is wider.

I would like to go over some of these aspects and viewpoints that are currently popular concerning legume seeds, because I think they should be more thoroughly analyzed and discussed.

1. *What are the perspective for legume seeds, considering production projections and their trade in the world?*

The latest FAO report (10) on the subject shows that production of the main legume seeds has been growing at a lower rate than that of the main cereals. Legume seed production has increased about 50% and that of rice and wheat have nearly doubled (Table 1).

When we look at the figures on the world demand for these foods as projected by FAO for the next 10 years (11) we see from these figures that there is a proportionally higher increase in the demand for the legume seeds than for wheat, and a similar increase in demand for rice (Table 2). This will result in a 1980 decrease in per capita demand for wheat and an increase for rice and legume seeds (Table 3).

TABLE 1

PRODUCTION OF LEGUME SEEDS*

	1948/52	1970
	Million metric tons.	
Legume seeds	29.3	44.4
Wheat	171.2	311.6
Rice	167.3	306.8

* FAO Yearbook 1970.

TABLE 2

TOTAL DEMAND PROJECTIONS (millions met. tons.)*

	1965	1970	1975	1980
Cereals	429	447	532	595
Rice	157	179	204	233
Wheat	151	165	180	198
Pulses Nuts Seeds	46	52	60	69

* FAO Agricultural commodity projections.

TABLE 3

LEVEL OF PER CAPITA DEMAND PROJECTIONS*
Kg/year

	1965	1970	1975	1980
Cereals	127	128	129	130
Rice	47	48	50	51
Wheat	45	44	44	43
Pulses, nuts	10	11	11	12

* FAO Agricultural commodity proyections, Rome 1971.

It is important to note that the demand for legume seeds has been 3 or 4 times less than that for wheat and rice, and the projections say it will be kept at these levels. These data are in accordance with the prediction of Roberts (1) that the conventional sources of protein in the diet will not change in the next 25 years. Again, if these figures turn out to be correct, a low increase in world production of legume seeds and a high total demand for a small per capita increase give us a poor perspective for legume seeds being of further importance as a world supplier of protein for human feeding.

When we look at world trade the perspectives are also not too favorable for legume seeds. FAO figures for imports and exports of this food as compared to wheat, a major item in this area, and to rice, which has a much smaller market, do not predict great progress for the legume seeds either in quantity or in economic value. These values can be higher in local market trade, but the problem still remains: how to make legume seeds a reasonable cash crop of importance in the world market.

TABLE 4

TRADE OF LEGUME SEEDS (100.000 metric tons.)*

	IMPORTS		EXPORTS	
WHEAT	657	646	563	568
Rice	78	75	79	73
Legume seeds	19	18	22	18

* FAO Trade Yearbook 1971.

2. *Improvement of diets deficient in protein could be achieved by the improvement of productivity, adaptability and yield stability of legume seeds.*

This approach was given the highest priority by the Protein Advisory Group of the United Nations in 1973 (12).

The implication is that a high input on the production of legume seeds would have a direct effect on protein nutritional status of needy populations.

There have been some reports that would support this asumption. Consumption of legume seed is higher at harvest time when they are more readily available (2). On the other hand, higher production of legume seeds has always been confronted with poor storage facilities, low prices, and marketing problems, with a result-

ing spoilage of the product, which ends many times to be used as fertilizer.

3. *Protein hunger may be largely overcome by an increase in availability of animal or plant protein, at price levels the affected population can afford.*

Legume seeds would be a reasonable tool with which to deal with the problem because, on the protein per price basis, legumes and particularly soybeans, are among the cheapest available sources of protein. The fallacy of this reasoning lies in the assumption that the needy people operate within the boundaries of the money economy and reasoning. Several times they do not buy and when they do, they give preference to their local staple food based on social and cultural context. It is not to be expected they will buy food because of its protein price or nutritive value.

Programs of protein food fortification and commercialization will always raise the problem of their impact on a practically out-of-the economy target group. It has been predicted that this approach could even be a factor that would decrease the nutritional condition of the needy people (13).

4. *Consumption of legumes can be easily increased because they are already eaten by people everywhere.*

It is true that certain amounts of legume seeds are eaten by millions of people around the world. But we should not be so optimistic as to think that just because someone is today eating 50 g, he will be eating 100 g tomorrow just because it is a source of protein. Social and cultural aspects, to say nothing of economics will certainly be factors to consider before we see a larger consumption of legume seeds by the people with the greatest protein needs.

5. *Higher consumption of legume seeds will increase the nutritive value of poor people's diets.*

It has been said that legume seeds are already consumed by poor people around the world. A better supply could result in a larger intake and consequently a better protein status. But it is well know that legume seeds are poor sources of sulphur amino acids. A higher intake of them does not necessarily mean better protein status. If a reasonable amount is already eaten, would it not be advisable to balance this diet with another source of protein?

Another aspect of the same subject was raised by Aykroyd and Doughty (2) when they reviewed the subject of legumes in human nutrition. They asked what was considered a key question on the subject: How much would a greater supply of legumes in developing countries improve the nutritional value of local diets? They stated that 30 g of legume added to a cereal diet would considerably raise its protein value, both for adults and children. Addition of 45, 60, or 90 g did not show a proportionally greater increase. They concluded that there would not be great advantage in larger consumption of legumes than is now customary in many tropical and subtropical areas.

Although I am not sure that we can generalize this statement, it is no doubt a warning against the assumption that higher legume intake will necessarily result in a better protein status.

6. *Legume seeds are low yielding crops.*

This is said to be one of the main reasons why legume seeds are not widely grown and consequently are not commonly used as a human food. The need for a green revolution for legume seeds has been said to be an immediate need.

How low yielding are legume seeds, and how far are we from green revolution levels?

Average world yields for most of the legume seeds range from 0.3 to 1.2 metric tons per hectare, as against 1.5 to 2.5 for the cereals and even higher levels with the new varieties. It must be said that as opposed to cereals, most of the agricultural practices with legumes are quite backwards, not mechanized and not fertilized (with the exception of soy, where higher yields are obtained). It is also known, and we have local results in Brazil, that common beans yields in poor soil jumped from 500 kg/ha to 2500 kg/ha with the use of adequate fertilization. Can this be considered a legume green revolution? At the CIAT meeting on legumes (14) it was said that yields as high as 4000 kg/ha have been obtained. It seems to me that the potential and knowledge for high yield legumes are here. There must be other factors that impede the practical utilization of this knowledge.

In relation also to the statement that legumes are a low yielding crop, Munger (15) states that this misconception has to be challenged. He proposed reporting yield not only on an area basis but also on a per unit time basis. In temperate climate conditions only one vegetable crop can be grown a year, but

in the tropics the land can be cropped continuously and a time element should be added in reporting yields. There seems to be little doubt that with available irrigation and high fertilization, yields of legume crops would certainly reach very high levels.

Another suggested approach to the problem would be to consider the nutrient value of the crop. This has been done with the legume seeds, and it has been shown that when the protein content and the nutritional value of the protein are taken into consideration, the average yield of protein nutrition from the legume crops approaches that of the cereals, even with a much lower yield per area (15).

7. *We can meet the protein needs of the poor in the poor countries with a poor man's foods, such as legume seeds.*

I would say this is a poor approach to the problem, and I think we should be more objective and practical about the subject. I am not going into the discussion that we should have a special food for low income groups in developing countries, or that the trickle-down or trickle-up theory is the best one (13). It is my suggestion that we should work with beans because of their potential for production, commercialization and consumption everywhere, both in the developing and developed countries.

Fortunately a boom in the human consumption of soy protein in the United States, with a predictied expansion of the market from 75 million dollars today to 2 billion dollars by 1980 (16) will point out that legume foods can also be part of the rich man's diet. It is also important to know that processing developments are in progress, making it possible to utilize other legume protein seeds to produce texturized products. This will certainly open a better and needed marketing for some of the other legume seeds (17).

8. *Legume seeds, actually used mainly as human food, have a large potential as animal feed.*

One of the problems with a large production of some legumes is their direct utilization only as human food. This will no doubt restrict its practical importance, and is one of the reasons that we include the studies of legume as animal feed in our meeting. These crops cannot be solely dependent on human consumption, and there is no reason for it not to be utilized by animals. If it is a good protein for humans it has to be good for the animals too.

With larger production, in order to be economical you have to industrialize it and also to direct it both for human and for animal feeding. Here again I see a broad perspective for marketing and utilization of larger supplies of legume seeds.

9. *Food technology will improve legume consumption through better convenience foods.*

Storage and industrialization are major problems obstructing the consumption and utilization of legume seeds.

Little is know regarding what adequate facilities for large legume seed storage would consist of. Aged beans are more difficult to cook and their acceptance is decreased. The creation of industrial processes which would reduce cooking time or make available precooked products would certainly increase utilization of these legumes.

Isolating and concentrating protein from legumes would certainly be another way to better utilize the legume protein. These processes would destroy the toxic factors. They would also reduce the high bulk needed for a high protein intake from the whole legumes. This would certainly be useful for infant and small child feeding.

10. *Practical application of research findings.*

A last point of importance is how to utilize research findings. I would like to reapeat here some of the words of Munger (15) concerning improved varieties of seeds. He says that the work put into them will be in vain unless the seeds is produced in large quantity, widely distributed and used. Too often in the tropics this final step has not been taken and the research might as well not have been started.

We have to be quite aware of this situation in relation to legume seeds. And here the use should not mean only the crop utilization of an improved seed but also what to do with the legumes after harvest. It is also important to emphasize the need for in-country and local programs with legume seeds, especially considering their large variety, economics and socio-cultural aspects. Research and applied programs have often suffered because people know a lot on the subject in a specific enviroment but their knowledge is not applicable elsewhere. Hybrid seeds are great and high yielding, but how can they be made available yearly in a backward region of a less developed country? High fertilization

was known to maximize agricultural production, but high costs and pollution problems resulting from its use has to make us consider other possible solutions. What about, for example, having local seeds bred for their ability to yield well at low levels of fertilization, as was attempted several years ago (15).

In summary, I would like to stress that a better perspective for the legume seeds will be reached with a broad approach to the subject including simultaneous work on production, marketing, industrialization, consumption and utilization. This approach should include consideration of both human and animal feeding.

I do not believe that any isolated work in legumes on only one aspect, such as increased agricultural production, or the consideration of their high protein content, of their low priced protein, or of being a food protein for poor people along with humanitarian reasons would allow, by itself, for the success of legume seeds as a protein supplier for the world today or in the future.

SUMMARY

The perspectives of legume seeds as food and feed are reviewed considering the need of a more broad and coordinated effort to deal with the problem. Aspect of legume production, improvement of diets through legumes, higher consumption of legume seeds by humans industrialization and practical application of research findings are discussed.

It is stressed that a better perspective for the legume seeds will only be reached through a broad approach to the subject including simultaneous production, marketing, industrialization, consumption and utilization. This approach should also include consideration for both human and animal feeding.

It is not believed that any isolated work in legumes on a particular aspect, such as increased agricultural production or the consideration of their high protein content, their low priced protein, or of being a food protein for poor people along with humanitarian reasons would allow, by itself, the success of legume seeds as a protein supplier for the world today or in the future.

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LEGUMES AS FEED FOR ANIMALS

1. *State of knowledge*

Recognizing that animal production is nutritionally important as a source of high quality protein food for human consumption and also economically important in terms of national development (e. g. export), the use of legumes for animal feed should be seen in the light of the potential and final impact on human needs.

The priorities in animal nutrition should be directed to recognizing that animals (particularly monogastrics) represent a significant vanguard mechanism in identifying and introducing new feed legumes, which with experience in animal feeding could evolve into human food sources.

At the present time in the Americas, soybean and peanut meals are the principal legumes used in animal feeding, because they are available as by-products of oil extraction and are the only ones with improved nutritional and economic characteristics. Also their nutritional properties have been investigated more thoroughly than that of other legumes. Although several other legumes are important as a source of protein for humans, little scientific information is available on nutritive value and possible use as feed for animals.

2. *Value of present knowledge and its use in practice*

The knowledge we have about the nutritional value of legume protein, principally soybeans, has been gained from animal studies. Soybean meal has formed the base for development of important animal products such as meat, milk and eggs for human consumption. This knowledge is being used in the preparation of high quality protein foods for direct use in humans. Other legume proteins which are less acceptable as food for men may be used effectively for producing animal protein foods of high quality. This contributes to our overall objective or goal of improving the diets for people especially the protein intake.

The direct diversion of legume seeds from human consumption should be avoided for products that are produced primarily for human food use. However, the by-products (hulls) should be

used in animal feeding. In times of surplus production the excess should also be used as animal feed, which will result in protein products of high value and high acceptability. In order to make the most effective use of legume seed protein in animal feeds, we must have more complete information on nutrient composition, nutrient utilization, presence of inhibitors or toxic factors and necessary processing and treatment. This same information is also important in using these products as human food.

3. *Recomendations*

Legume seeds other than those now produced primarily for human food should be investigated as sources of protein for animal production from the following points:

a) Adaptability for feeding animals in different regions.

b) Yield of protein in comparison with other crops.

c) Utility of the entire plant (seed and straw) as animal food.

d) Potential for eventual use of new legume as food for animal, for example *Clitoris ternata* and *Siratrum*.

e) Amino acid composition, presence or absence of inhibitors and the processing requirements for use in feeds.

f) Compilation and publishing of available information on feed values, including different areas, such as economics, social, nutrition, etc.

STORAGE, PROCESSING AND NUTRITIVE VALUE OF LEGUMES

1. *State of knowledge*

Based on material presented by speakers during Session II and on other published inform ation the following conclusions are made:

a) Moisture content of grain legumes and storage temperature have been shown to influence nutritional value, processing characteristics, and ease of cooking of beans.

b) Storage of beans at moisture level of 12 to 16% caused an increase in cooking time and a lowered nutritional value in comparison with beans stored at 8% moisture.

c) Stored beans with a 12-14% moisture and at a temperature of about 5°C were superior for processing or production of a precooked bean powder than beans stored at a higher temperature.

d) Storage temperature and moisture have an important influence on the proportion of "hard shell" beans.

e) Improved storage facilities and processing (drying, cleaning, etc.) prior to storage are essential to preserving grain legumes

and their nutritional value and for preventing losses from insect infestation, microbial growth (molds, bacteria) and from rodent consumption and contamination.

f) The use of insecticides is effective in preventing insect losses but better and more acceptable insect control methods are needed, now that DDT is not available.

g) Beans and other grain legumes can be processed to produce products of high nutritional value and good acceptability, but the added cost of such processing can be a deterrent to use of such products by low income groups, unless subsidized by governments.

h) Some legumes which are well adapted to areas or regions but have poor acceptability as human food or are of low nutritional value can be used in the preparation of protein concentrates of high value and acceptability for many food uses. An example is a protein concentrate made from jack beans *(Canavalia ensiformis)*.

i) Some of the grain legumes have been shown to respond very differently to cooking treatments and to have nutritional values much higher than common beans *(Phaseolus vulgaris)*. Examples are cowpeas and lima beans *(Vigna sinensis, Phaseolus lunatus)*. The soybean appears to have the highest nutritional value when properly cooked.

j) Methionine appears to be the most limiting amino acid for all the grain legumes but in some instances tryptophan is also limiting i. e. for the pigeon pea *(Cajanus cajan)*.

:) The use of reducing sugar in formulations of food products containing beans or legume proteins should be kept to a minimum or isolated from proteins, especially when the products are to be heated or cooked after the sugar is added. The sugar reacts especially with lysine to destroy it or make it unavailable.

2. *Value of present knowledge and its use in practice*

Our present knowledge related to the subject is extensive and we have a well developed technology on storing grains, processing grains before and after storage, but this is related to aspects other than nutritional value. We also have a limited amount of knowledge to help explain the basis of some changes that take place in nutritional value during storage of beans and during maturation of seeds and in germination. Some processing treatments have resulted in unexplained improvement of nutritional value of beans and in some cases cooking treatments have not given the expected responses.

The available knowledge is being used to very different degrees

in different countries and under different circumstances. The technology is being used in many cases by adapting it to local conditions and with local products. In many cases the use of knowledge and technology is not possible because of the lack of suitable structures, facilities or equipment. This is especially true in relation to proper storage of beans and other grain legumes to minimize losses and deterioration in nutritive value. In other cases the available knowledge is not effectively used because of the failure to provide the information in an understandable form and to the people who need it.

3. *Recommendations*

Programs of research and development should be organized and implemented that are appropriate for and especially suited to different countries or regions. These programs are needed to make maximum use of current productions and to enhance nutritional values, acceptability and processing properties of beans and other legumes.

Investigators are urged to clearly describe the experimental material giving the type of product used, basis of analysis, variety (or code number), genus, species, date of harvest, area of production, cooking methods and any other available information that would be useful in the interpretation of results.

The following specific suggestions are made for consideration:

a) A comprehensive national policy based on the best knowledge available be approved by each country to ensure production of needed products and to provide necessary and suitable storage facilities for the production. This type of policy should also ensure that the production would be available for domestic needs.

b) Cooperative programs between governments and the private sector be implemented to develop new processes and products utilizing beans and other legumes to enhance or improve the nutritional status of the people.

c) Research programs should be supported to investigate the basis or mechanisms involved in the deterioration of the nutritive value of beans during storage.

d) Conduct work to compare effectiveness of storage systems for other grains in storing beans and other grain legumes and design or devise facilities and storage systems that may be suited to use by small producers: for example, plastic bags, drums, modified atmosphere, underground storage, etc.

e) Investigate further the influence of variety, storage con-

ditions, stage of harvesting, method of drying, etc. on cooking time, acceptability, nutritional value of beans, content of natural toxins and the development of micotoxins.

f) Screen or test large numbers of varieties and types of legumes to determine their adaptability to a region or country and compare their nutritional value with those already being produced, to identify superior germplasm for use in breeding programs and in production.

g) Investigate the possibility of utilizing legumes that are indigenous or well adapted to a region but not currently produced commercially in production of products of high nutritional value for use either in animal production or as human food. This might be accomplished by preparing protein isolates or by special processing to decrease toxicity or increase nutrient availability.

h) Develop more effective extension or adult education programs to diseminate new information and to gather information on local customs, practices and products that might be useful in improving storage, processing and utilization of beans and other legumes.

i) Investigate further production, acceptability, marketing and performance of low cost foods including snack foods of high nutritional value prepared from beans and other legumes.

COMMON BEANS AND OTHER LEGUMES IN HUMAN NUTRITION

1. *State of knowledge*

It was pointed out that previous publications on the subject such as the FAO report of 1966 and the PAG statement Nº 22 are useful references.

The following points were considered of importance:

a) Even though the group considered that a complete review of legumes was difficult to accomplish, regional and withincountry differences were considered. The reasons for this differences were explained by different hypotheses. Evolutionary, adaptive and selection mechanisms probably had influences in original choices. Other factors appeared later, linked to human adverse factors and organoleptic characteristics.

b) Complementation of cereals by legumes has been used by societies every-where throughout history. The Green Revolution has incerased the availability of cereals without a simultaneous concern for legumes. It is of great importance to increase legume

consumption by extension of the Revolution to this staple. Furthermore, legumes with their high protein content could more efficiently than cereals help to narrow the protein gap. Even though the science of nutrition itself may not have had an influence on the habits, it has been shown that people will tend to combine foods in the best proportion if all components are available. It is reasonable to believe that increased legume consumption should be stimulated.

c) The latter suggestion posed another related problem which was considered by different groups as the most important: to increase availability of legumes both by increased production and decrease of losses and prices. The development of appropriate storage facilities at the production site and for industry could permit a more permanent supply and less price fluctuations.

2. *Value of present knowledge and its use in practice*

It is necessary to create public awareness and a greater general appreciation of the nutritional value of legumes. Marketing systems or traditional extension program and the design of new forms of preparation (at home and commercially) could break barriers for the use of unfamiliar varieties and methods.

Genetics or food engineering approaches may be necessary to resolve certain problems such as cooking time, toxicity and flatulence, which could set the ceiling for consumption.

It is strongly suggested that there is a great need to expand the basic support for application of knowledge, to influence policy makers and planning officers of a country.

The suggestion was made for more systems analysis to prove or disprove the public benefits of expanded legume production and consumption.

3. *Recommendations*

a) Increase production and improve quality.

b) Storage facilities for both small and large producers.

c) Study systems of marketing and advertising to create public awareness, increase demand, promote indigenous and unfamiliar varieties.

d) Study the ways, genetical or technical, to decrease adverse factors.

e) Study systematically the cost-benefit effects of expanded legume production, taking all factors into consideration.

f) Study mechanisms to influence policy makers and government agencies for the application of the known facts.

Analitical methods for the study of nutritional qualities of beans

At the conclusion of the symposium a group of 20 participants met to discuss priorities in studies of factors or properties related to the nutrional value of beans. Some twenty one pertinent factors were subdivided into four groups in terms of physiological importance and ease of determination. This preliminary classification should be revised periodically on the basis of new research results. It was proposed that available analytical methods, as well as the known nutritional aspects of these factors be compiled and distributed to all those interested. It was further considered that standard bean samples be made available to research laboratories through agronomical centers such as CIAT in Cali, Colombia. These standards should be universally employed as a basis of comparison of future experimental results.

It is desirable that laboratories, preferentially with previous experience, would be dedicated to the routine analyses of certain bean factors of nutritional importance and offer their services to plant breeders for assaying pre-selected samples of satisfactory agronomical performance. Funds for financing this program should be established. It was generally felt that a program of this nature might contribute to the improvement of the nutritional value of new lines of bean varieties and also benefit industrialized bean products. This program would further assure that assays and interpretation of experimental results be made by highly specialized and most qualified research groups. Moreover, overall expenditures would be considerably cut down, since financing the setup of a large number of diverse methods in any one laboratory is prohibitive.

PRIORITY GROUPS FOR THE DETERMINATION OF NUTRITIVE CHARACTERISTICS OF BEANS

GROUP	I	Cooking characteristics Total protein content Amino acids (methionine, cystine, tryptophan, lysine) Sulphur Hemagglutinins
GROUP	II	Protein digestibility Digestible energy PER, NPU Organoleptic characteristics
GROUP	III	Protease inhibitors Amylase inhibitors Flatulance factors Available aminoacids
GROUP	IV	Phytic acid Pectic compounds Oligosacacharides Vitamins Fe HCN